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Morphology and Functional Changes of Intestine, Trophology Status and Systemic Inflammation in Patients with Chronic Heart Failure

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1. Introduction

1.1 Morphological and functional changes of the small intestine in patients with different classes of chronic heart failure

Current understanding prompts to view chronic heart failure (CHF) as a systemic condition. Traditionally, the following organs are considered the target organs of CHF: heart, kidneys, brain. However, low cardiac output and increased activity of the renin-angiotensin-aldosterone system (RAAS), which lead to vasospasm and ischemia, are bound to have effect on functions of other organs, including small intestine, large intestine, and adipose tissue. The increased activity of RAAS is likely to have effect on morphological restructuring of the intestine as well. It can be assumed that accumulation of collagen in the intestinal wall, as well as development of edema and ischemia, decrease the functional activity of the intestinal wall and are a major factor of the malabsorption syndrome, which, in its turn, leads to progressive loss of body mass. This results in progression of certain clinical manifestations in patients with CHF, such as: weakness, fatigue, and progressive decrease of exercise tolerance, which cannot be explained by changes in peripheral circulation alone. The incidence of these complaints is known to increase as the NYHA class of the CHF grows, and it reaches its peak in patients with class IV (Harrington & Anker, 1997).

Loss of body mass means a significantly worse prognosis for the patients with CHF. According to SOLVD study, ≥6 % decrease of body mass in patients with CHF is a potent predictor of negative impact on survival along with other factors such as age, gender, LV ejection fraction, NYHA class (Anker et al., 2003).

Therefore, decrease of body mass should be considered an important sign, equal in significance to such symptoms as dyspnea and edema.

Obviously, a search for new methods to correct the nutritional status in patients with CHF is necessary. The method of nutritional support may be one of these promising options to treat and stop development the malabsorption syndrome, as this method constitutes a system with pathogenesis-based rationale that implies prescription of balanced nutritive mixtures

characterized by maximum degree of absorption even in the setting of morphological changes in the intestine.

1.2 Materials and methods

This was an open-label prospective study approved by the Ethics Committee of the Russian State Medical University. All of the patients participating in this study had signed the informed consent.

The study included 110 patients with New York Heart Association (NYHA) class I-IV ischemic CHF, with history of CHF for more than 24 months. Age of the patients was 45 to 65 years, mean age was 58.7±5.3 years. All of the patients were allocated to different groups based on the severity of their CHF. The control group included 38 patients (24 male and 14 female), 45 to 65 years old (mean age 60.6±2.6), in which no signs of CHF were found after testing. The patient populations had comparable basic parameters at baseline.

All of the patients with the signs of CHF received standard basic therapy, which included ACE inhibitors, β-blockers, loop diuretics or thiazide diuretics, cardiac glycosides, nitrates, aspirin, and aldosterone antagonists. Patients in the control group received therapy for their main condition.

Patients were investigated using the following sets of tests:

- Endoscopy with biopsy of the small intestine (the samples were taken 10 cm below the plica duodenojejunalis). Preparation and analysis of microscopic specimens were performed in the Pathology Department of the Bakulev Scientific Center of Cardiovascular Surgery.
- Assessment of functional activity of the small intestine (measurement of excretion of fat in feces, biochemical measurement of total protein and protein fractions in feces via nitrogen content, measurement of carbohydrate absorption in the small intestine using the D-xylose test).

Statistical analyses of the results were performed using standard statistical formulas with Microsoft Excel 7.0 and BIOSTAT software. Arithmetic means of values in the sample population (M) and standard deviations (σ) were determined. The significance of differences between groups was determined using Student test at $p < 0.05$. Relationship between parameters was assessed by calculating the correlation coefficient (r) with the level of errorless prognosis at 95 % ($p<0.05$).

1.3 Results

1.3.1 Morphological changes of the small intestine in patients with NYHA class I-IV CHF

The photographs of microscopic specimens (Fig. 1) demonstrate the pattern of the mucosa of the small intestine typical for patients with NYHA class IV CHF (a) and healthy individuals (b). Collagen fibers are stained pink.

These photographs demonstrate that in patients with NYHA class III-IV CHF the collagen fibers stained pink take up a significant area of the small intestine mucosa, while in patients without signs of CHF only solitary collagen fibers are present.

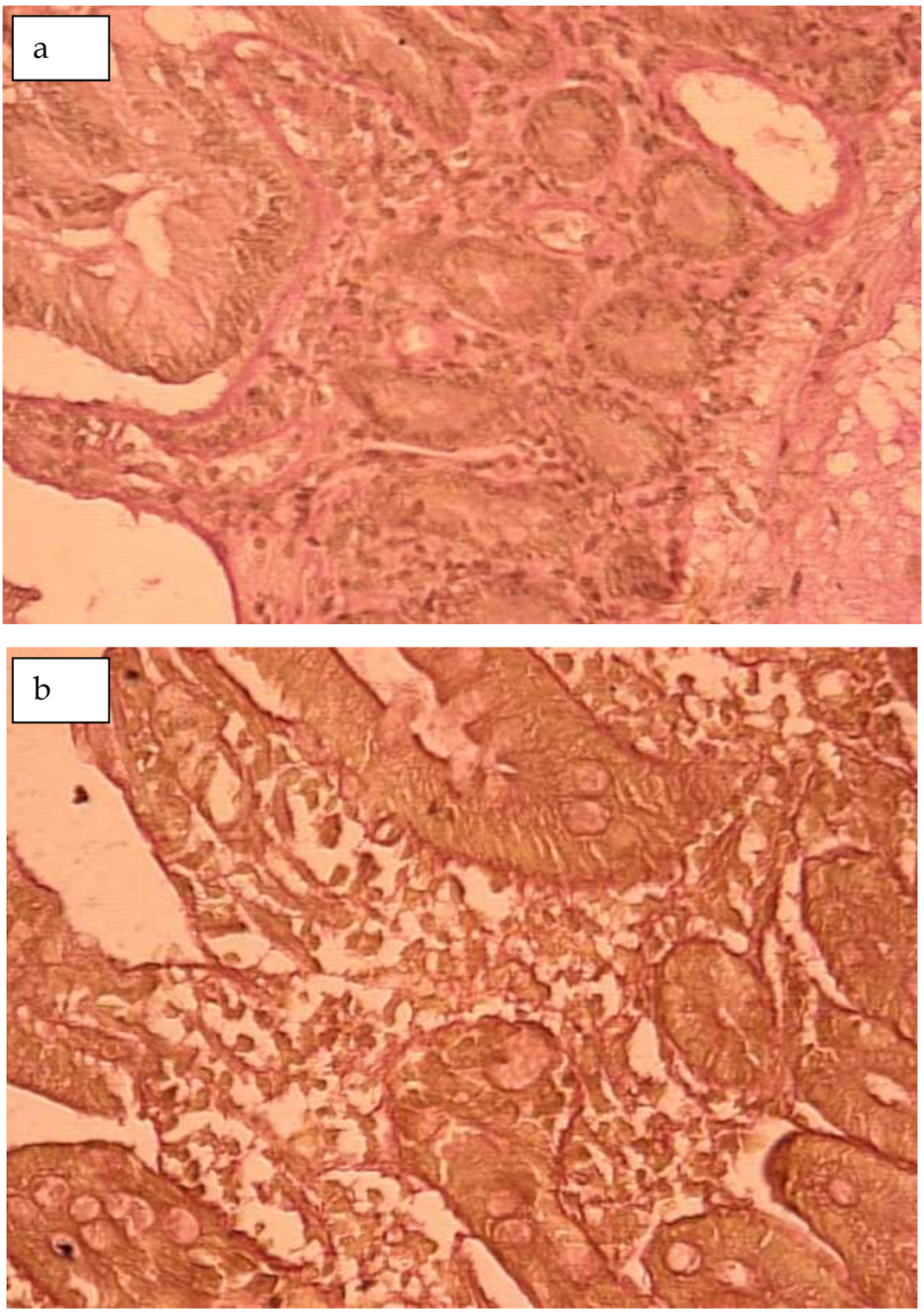


Fig. 1. The microscopic sample of the mucosa of the small intestine from a patient with NYHA class IV CHF (a) and a patient without signs of CHF (b). Van Gieson's stain, magnification x 400.

Collagen deposition level in patients with NYHA classes III and IV was significantly different from values measured in patients with NYHA classes I or II and in patients without signs of CHF. Comparison of collagen relative density between patients without CHF and with NYHA class I or II CHF did not reveal significant differences. However, a clear trend towards increase of collagen level in the latter was noted. Also, the difference in the amount of collagen between CHF patients with NYHA classes III and IV was not significant.

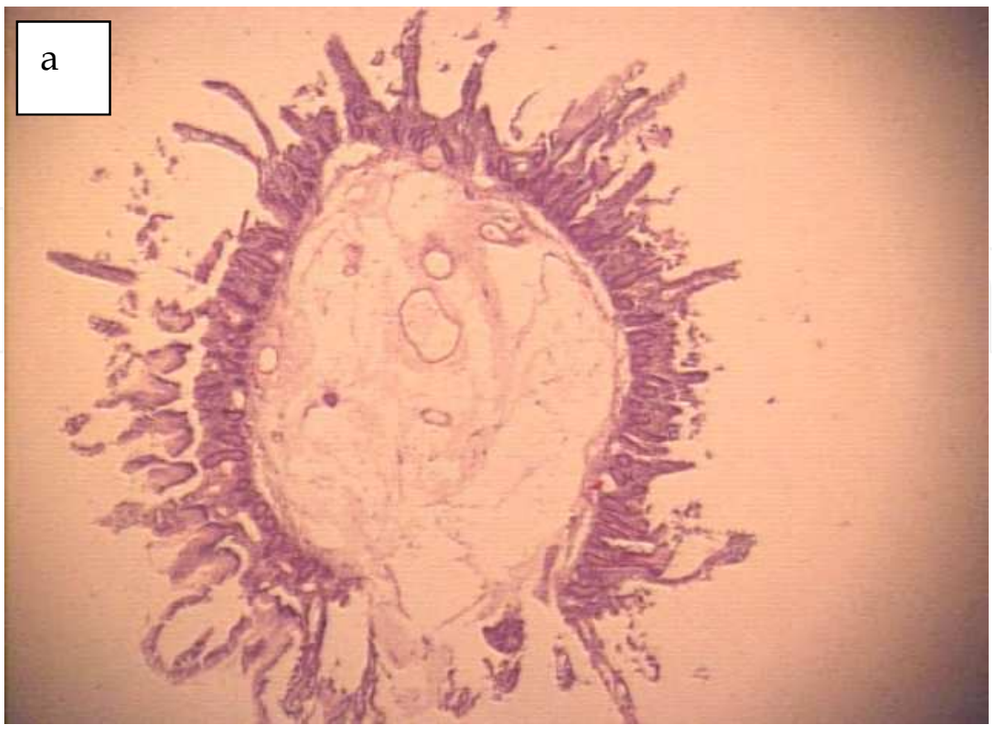
When assessing the microscopic specimens of the small intestine, high amount of collagen was observed to mechanically push enterocytes away from a capillary vessel. This increases the intestine-blood barrier and may have an impact on nutrient absorption.

The measured data are presented in Table 1.

Groups	Distance between EBM and a capillary vessel, μm	Significance of difference between groups
1. No CHF	8.4±0.7	p1-2 >0.05; p1-3>0.05; p1-4<0.05; p1-5<0.05
2. NYHA class I	10.1±1.2	p2-3>0.05; p2-4>0.05; p2-5>0.05
3. NYHA class II	11.3±1.1	p3-4<0.05; p3-5<0.05
4. NYHA class III	18.6±1.4	p4-5>0.05
5. NYHA class IV	19.1±1.2	

Table 1. Distance between EBM (enterocyte basal membrane) and the capillary wall

Even primary assessment of microscopic photographs revealed atrophy of villi of the small intestine mucosa in patients with NYHA class III-IV CHF. Fig. 2 shows pattern of the mucosa in a patient with NYHA class IV CHF (a) compared to a patient from the control group (b).



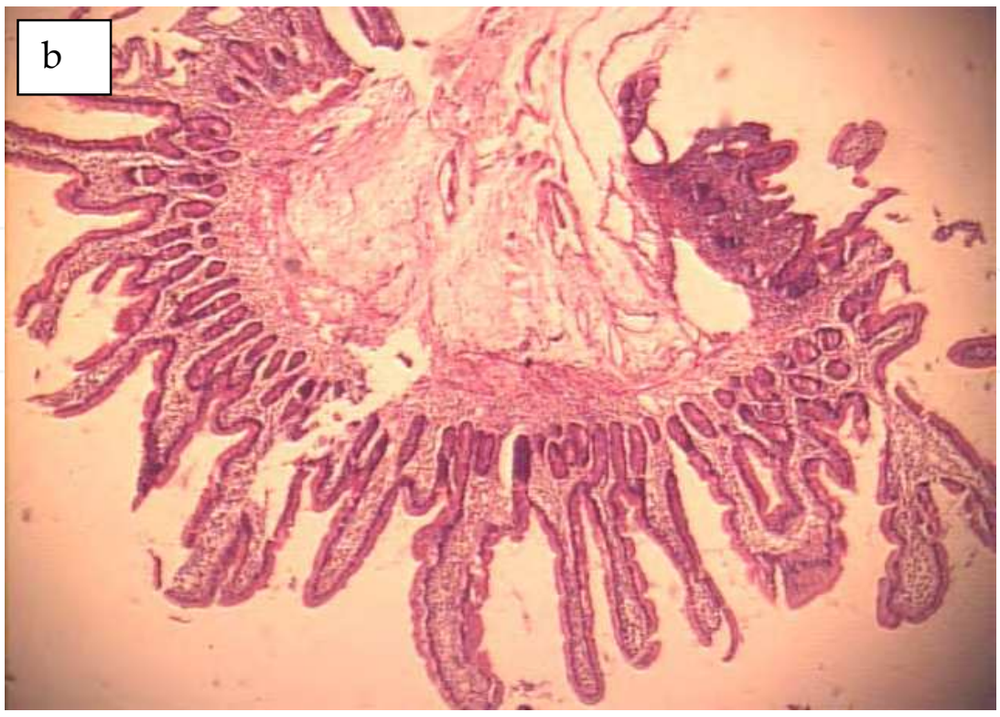


Fig. 2. Villi of the small intestine mucosa in a patient with NYHA class IV CHF (a) and a patient without CHF (b).Magnification x100. Hematoxylin-eosin stain.

Quantitative values describing changes in the villi of the small intestine in patients with different classes of CHF are shown in the Table 2.

Groups	Mean length of a villus, μm	Mean width of a villus, μm	Significance of difference between groups
No CHF	372±9.9	98±4.4	p1-2>0.05; p1-3>0.05; p1-4<0.05; p1-5<0.05
Class I CHF	369±7.4	94±3.5	p2-3>0.05; p2-4>0.05; p2-5>0.05
Class II CHF	374±6.9	91±6.1	p3-4<0.05; p3-5<0.05
Class III CHF	254±5.5	65±3.1	p4-5>0.05
Class IV CHF	223±6.1	59±3.0	

Table 2. Length and width of mucosal villi in the small intestine of patients with CHF.

To conclude, analysis of the small intestine mucosa biopsy samples demonstrated significant morphological changes, which worsen with the severity of CHF. Increase of intestine-blood barrier and decrease of the absorbing area of the villi determined further study of nutrient absorption, which, supposedly, should have decreased significantly in patients with CHF.

1.3.2 Functional changes of the small intestine in patients with NYHA class I-IV CHF

Analysis of protein absorption parameters revealed the following pattern: in patients with NYHA class I CHF, losses of total nitrogen were virtually below the upper limit of normal range, reaching 7.1±0.2 % of daily consumption. No significant differences were found

between the patients of the control group (5.9±0.21 %) and classes I or II ($p>0.05$). With NYHA class IV CHF, the loss of protein was significantly higher – 18.6±1.3 %; this was on average 3.1 times higher than in the group of patients without CHF ($p<0.05$). In the group of patients with NYHA class III, the loss of protein was 16.7±1.8 %. Comparison of the protein loss levels in CHF patients with NYHA class III and IV vs. the protein loss levels in patients with NYHA class I and II showed a significant difference ($p>0.05$).

Levels of total fat loss were most pronounced in CHF patients with NYHA class III and IV (22.4±2.1 % and 24.1±2.12 % of the daily consumption, respectively) and exceeded the levels in patients without CHF (5.5±0.86 %) 4-fold on average. For NYHA classes I and II, fat loss levels were at the upper limit of normal (6.1±1.1 and 7.2±0.9 % of daily consumption, respectively) and were not significantly different from the values in the group of patients without CHF ($p>0.05$).

Analysis of D-xylose test results demonstrated a dependency similar to the one observed for absorption of proteins and fats. In patients with NYHA class IV CHF, the 5-hour excretion of D-xylose was 0.89±0.05 g. This was 1.4 times lower than the values for the control group. For NYHA class III CHF, D-xylose excretion was 0.96±0.03 g. This was also significantly different from normal values. Patients with NYHA class I and II did not show significant differences compared to the control group.

To conclude, patients with CHF experience deterioration of absorption for all basic nutrients, and the absorption reduction demonstrates dependence upon the severity of the CHF.

1.4 Attempts for correction

Taking into consideration the pronounced changes of the small intestine in patients with severe CHF that lead to development of malabsorption syndrome and protein-energy insufficiency, such patients demand specific correction of their nutritional status. Naturally, raw nutrients will hardly be utilized in the intestine that underwent these changes. A possibility to use specifically treated nutrients in the form of standard mixes for oral feeding was the objective that we assessed in the second phase of this study.

This part was an open-label, randomized, prospective, 24-week study.

Patients with NYHA class III and IV CHF were screened for this study. Total number of subjects was 74 (46 males, 28 females).

Randomization was performed using a random number method, with even numbers corresponding to the standard-of-care group (Group 1), and odd numbers corresponding to the nutritional support group (Group 2). The number of patients assigned to Group 1 was 36. Group 2 included 38 patients.

Figure 3 shows the study design.

Subjects in Group 1 (n=36) received standard basic therapy and their usual nutrition within the standard diet for cardiovascular patients.

Subjects in Group 2 (n=38), in addition to the basic therapy and standard diet, received a balanced nutritive mix, comprising 25 % of daily calories.

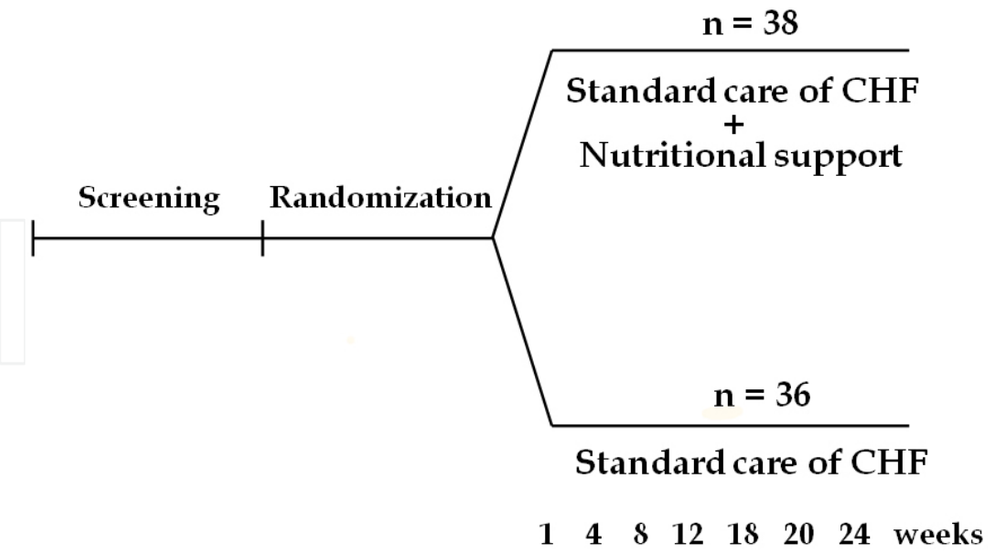


Fig. 3. Study Design.

The absolute majority of the patients were male and had history of ischemic origin of their disease. Their mean age was over 60 years. Mean NYHA class within these groups was 3.5 and 3.4, respectively. The resulting groups were identical in structure by gender, age and other clinical characteristics, warranting their comparability. The following tests were performed during this study: 6-minute test, echocardiography (LVEF), diet review, measurement of body mass and BMI, estimation of body fat mass and lean body mass, total protein, albumin, absolute lymphocyte count, hand dynamometry, assessment of absorption for proteins, fats, and carbohydrates, morphometric study of small intestine mucosa biopsy specimens, count of hospitalizations due to CHF progression.

Estimations of energy and nutrient demands were performed for all subjects before therapeutic diet and nutritional support were prescribed.

Subjects in Group 2 had 25 % of their energy demands (daily energy consumption) covered using balanced nutritive mixes, and the remaining 75 % were covered using the standard diet. All patients maintained dietary diaries, which allowed us to control the amount of energy they have received with their usual diet.

The balanced nutritive mixes used for nutritive support were Peptamen (Nestle, Switzerland), Berlamin Modular (Berlin-Chemie, Germany), Unipit and Nutrien-standard (Nutritek, Russia).

Assessment of nutritional support efficacy included change of 6-minute test parameters over time, change of LBM over time, and number of hospitalizations due to CHF progression compared between the experimental group and the control group.

During 24-week observation, a total of 11 patients died in 2 groups: 4 males and 2 females in Group 1 (standard-of-care), and 3 males and 2 females in Group 2 (oral nutritional support). Total number of hospitalization events throughout 24 weeks was 54 and 42 in Group 1 and Group 2, respectively. Table 3 shows causes of death and hospitalization.

Cause	Hospitalization		Death	
	Group 1	Group 2	Group 1	Group 2
CHF progression	36 (66,7%)	28 (66,7%)	4 (66,7%)	3 (60%)
Recurrent MI	6 (11,1%)	7 (16,7%)	1 (16,7%)	2 (40%)
Pneumonias	10 (18,5%)	6 (14,3%)	1 (16,7%)	0
Other	2 (0,4%)	3 (0,7%)	0	0
Total	54	42	6	5

Table 3. Causes of death and hospitalization for Groups 1 and 2.

Three patients chose to withdraw from study: 2 of them were from the standard-of-care group, and another one from the nutritional support group (the reason was moving to another city). Compliance was assessed using patient diaries, where their adherence to basic therapy and to nutritional support was recorded. Compliance below 80 % was reported for 2 subjects in Group 1 and 2 subjects in Group 2 (these subjects were excluded from the final analysis). As a result, in the standard-of-care group 26 patients completed the study, and in the nutritional support group 30 patients completed the study.

1.4.1 Changes of 6-minute test parameters over time

The trend for growth of the parameters in Group 2 was noticeable starting from Week 2. The curves of 6-minute test for the 2 groups diverged significantly starting from Week 8. After 24 weeks, the exercise tolerance in patients receiving standard nutrition decreased significantly ($p=0.025$). The baseline values for 6-minute test in this group were 85 to 243 m, mean 203.4±41.6 m. After 24 weeks, the mean distance walked in 6 minutes decreased by 19 % (164.7±48.1 m).

Six patients with NYHA class III CHF experienced substantial deterioration of their health – worsening of dyspnea, weakness, edema, i. e. their condition progressed to NYHA class IV.

In the group of patients receiving the nutritional support, the baseline for the 6-minute test was 182.2±45.6 m (range 34 m to 221 m), and Week 24 mean was 231.3±41.1 m (75 m to 295 m), i. e. a statistically significant ($p=0.015$) increase in exercise tolerance was observed. In this group, progression of NYHA class III to class IV was recorded for 2 patients only.

To conclude, in patients receiving the standard diet, Week 24 exercise tolerance decreased significantly, whereas in patients receiving nutritional support, significant increase of the exercise tolerance was observed. Statistical significance for the difference between the groups was $p=0.021$.

1.4.2 Change of hand dynamometry measurements over time

After 24 weeks of monitoring, no significant differences were found for dynamometry variables when comparing pre-treatment and post-treatment values; however, there was a trend towards increase for these variables in the nutritional support group (mean increase 0.2 kg, $p=0.084$). In the standard-of-care group, the variables decreased, with mean change of 0.4 kg; this change was not statistically significant ($p=0.09$).

1.4.3 Change of LBM over time

For most of the patients receiving nutritional support, a significant increase of LBM was shown (mean change 5.8±1.2 kg or 8.9 %, p=0.038). For 2 patients, a progressive decrease of LBM was recorded on treatment (the LBM decreased by 2.1 kg and 3.4 kg). In the standard nutrition group, 23 patients experienced statistically significant decrease of their LBM (mean decrease 3.6±0.7 kg or 4.9 %, p=0.036). The LBM did not change significantly in 2 patients, and an increase of LBM (+1.7 kg) was reported for 1 patient.

To conclude, long-term nutritional support leads to statistically significant increase of LBM, while in the standard nutrition group the LBM continues to decrease progressively. The differences between groups were statistically significant (p=0.04).

1.4.4 Changes of laboratory variables over time (absolute lymphocyte count and serum albumin)

In the nutritional support group, a statistically significant increase of nutritional status was demonstrated: the absolute lymphocyte count increased from 1590 to 1710 x109 (+12.5 %, p=0.04), and the albumin level increased from 25.1 to 29.4 g/L (+17.1 %, p=0.045). In the standard-of-care group, no significant changes were observed after 24 weeks of monitoring for the lymphocyte count and albumin. The differences between groups were statistically significant (p=0.04).

To conclude, the nutritional support demonstrated a favorable effect for all basic nutritional status variables.

1.4.5 Other variables

For the effect of nutritional support on protein, fat and carbohydrate absorption variables see Table 4.

Absorption variables	Group 1		Group 2	
	Baseline	Week 24	Baseline	Week 24
Total protein loss, % of daily consumption	18.1±0.3	17.9±0.2	17.9±0.2	15.48±0.9
Total fat loss, % of daily consumption	22.5±0.6	21.6±0.4	23.4±0.4	20.7±1.3
D-xylose excretion with urine, g/5 h	0.81±0.05	0.85±0.04	0.82±0.04	0.83±0.04

Table 4. Absorption of nutrients in patients with NYHA class III-IV CHF, pre-treatment and post-treatment.

No statistically significant changes were demonstrated in either group for morphometric variables during the treatment period of 24 weeks.

1.5 Conclusion

The study of the small intestine condition revealed marked changes of structure and functional activity of the intestine in all patients with CHF. The degree of the small intestine

impairment directly depends on the severity of the CHF. This suggests direct impact of heart failure on gastrointestinal restructuring.

We consider the oral nutrition system involving prescription of balanced nutritional mixes to be one of the promising options to treat cardiac cachexia, as it has a pathogenesis-based rationale. This study was an attempt to evaluate the efficacy of the nutritional support during CHF progression.

2. Morphological and functional changes of the large intestine in patients with different classes of chronic heart failure

2.1 Introduction

Current understanding acknowledges the role of the following factors in the pathogenesis of CHF: neuroendocrine imbalance with excessive production of neurohormones, impairment of various target organs (cardiac muscle, kidneys, skeletal muscles, small intestine). However, many authors observed increased level of pro-inflammatory cytokines in CHF patients that cannot be explained by neuroendocrine activation. A number of authors reported a correlation between cytokine levels and blood plasma concentration of the endotoxin (lipopolysaccharide of gram-negative bacteria).

In an attempt to find the origin of the endotoxin, various bacteria were considered, particularly the bacteria of upper and lower respiratory tract, H. pylori, microorganisms of urinary tract and intestine. The strongest changes were found in the flora of the large intestine, where increase of the total number of microorganisms was observed, predominantly gram-negative. However, there were no reports of detailed analysis of the intestinal flora composition, and the parietal mucin layer flora was not taken into account.

The data on flora changes suggested potential methods of correction, particularly the selective decontamination method. However, according to literature reports, a course of selective decontamination reduces the number of microorganisms in the large intestine in the phase of antibacterial treatment only, while as early as 6 weeks after their discontinuation characteristics of the flora return to baseline. This shows lack of efficacy of the selective decontamination alone in CHF patients with high NYHA classes.

From our perspective, there are two possible approaches to correct the endotoxemia that leads to systemic inflammation: (1) development of methods that act on the intestinal wall by decreasing its permeability to the endotoxin, and (2) treatment that has direct effect on the intestinal flora towards normalization of both numbers and the composition of the flora.

2.2 Materials and methods

Laboratory tests.

Quantitative assay of endotoxin level using Kinetic-QCL test №50-650 U "Bioscience Cambrex Wallkersville", USA.

Quantitative assay of IL-6 (IL-6 Human ELISA Kit (1 x 96 Well Plate), Cytokine company, Russia), TNF-alpha (TNF alpha Human ELISA Kit (1 x 96 Well Plate), Cytokine company, Russia), CRP (C Reactive Protein Human ELISA Kit - 1 x 96 Well Plate, Abcam, USA) plasma levels using solid phase ELISA.

2.3 Assessment of the microbial landscape, large intestine wall structure, endotoxin levels, and pro-inflammatory cytokines in CHF patients with different NYHA classes

To study these variables, three consecutive groups were enrolled: Group 1: 65 patients with NYHA class III-IV CHF; Group 2: 60 patients with NYHA class I-II CHF; Group 3: 56 patients, control group (patients with ischemic heart disease and arterial hypertension without signs of CHF).

2.3.1 Changes in lumen flora of the large intestine in CHF patients with different NYHA classes and in the control group

Comparison of the first and the second study groups revealed statistically significant differences ($p<0.05$) for the following variables: total number of enterobacteria was 10^9 colony-forming units (CFU)/g in Group 1 vs. 10^7 CFU/g in Group 2. Enterobacteria pool growth was predominantly formed by *E. coli* (10^7 CFU/g in NYHA I-II group vs. 10^9 CFU/g in NYHA III-IV group, $p<0.0001$), various *Klebsiella sp.* (10^5 CFU/g in NYHA I-II group vs. 10^7 CFU/g in NYHA III-IV group, $p<0.005$), and citrate-assimilating enterobacteria (10^6 CFU/g in NYHA I-II group vs. 10^8 CFU/g in NYHA III-IV group, $p<0.005$). Differences in concentrations of *Citrobacters, Enterococci* and *Candida* yeasts were also statistically significant.

Comparison of the results for Group 1 (NYHA class III-IV) and control group subjects showed differences similar to the comparison of Group 1 versus Group 2, with the exception of differences in Clostridia populations (lecithinase- and hydrogen sulfide-positive strains): 10^7 CFU/g in CHF patients with NYHA III-IV vs. 10^5 CFU/g in the control group ($p<0.05$).

Comparison of Group 2 (NYHA I-II CHF) versus control group demonstrated minimal changes in the gut microbiome. Statistically significant differences were shown for *Bacteroides* only (10^9 CFU/g in NYHA class I-II patients vs. 10^{10} CFU/g in the control group, $p<0.05$). In CHF patients with NYHA class III-IV the levels of *Bacteroides* were not significantly different from results reported for the control group; therefore, from our perspective, these data can be ignored in practice.

Statistically significant differences were demonstrated for *Clostridia* (hydrogen sulfide-positive, lecithinase-positive): 10^5 CFU/g in the control group vs. 10^7 CFU/g in CHF patients with NYHA class III-IV, as well as for *Enterococci* and *Candida* yeasts ($p<0.05$). No statistically significant differences between groups were demonstrated for other microorganisms.

Conclusion: the higher is NYHA class of CHF, the stronger are the changes in the large intestine flora due to growth of gram-negative species.

2.4 Changes of the parietal mucin layer flora in CHF patients with NYHA class I-IV

The microorganisms located in the parietal mucin layer have the most significant impact on the host. Therefore, our objective was to study the changes in parietal mucin layer flora in CHF patients with NYHA class I-IV.Taking into account the minimal changes in lumen flora between NYHA class I-II patients and the control group, and considering technical difficulties associated with parietal flora studies, we decided to skip investigation of parietal mucin layer flora in the large intestine for the control group.We decided to approximate the results from biopsies of CHF patients with NYHA class I-II as normal.

Biopsy studies showed changes similar to those reported for feces. A statistically significant changes of the enterobacteria population in CHF patients with NYHA class III-IV was demonstrated (10^8 CFU/g vs. 10^5 CFU/g in patients with NYHA classes III-IV and I-II, respectively; $p<0.0001$).These changes were due to growth of *E. coli* (10^8 CFU/g vs. 10^5 CFU/g in NYHA III-IV vs. NYHA I-II, respectively; $p<0.0001$), *Klebsiella* (10^8 CFU/g vs. 10^5 CFU/g in NYHA III-IV vs. NYHA I-II, respectively; $p<0.005$), and citrate-assimilating enterobacteria (10^8 CFU/g vs. 10^5 CFU/g in NYHA III-IV vs. NYHA I-II, respectively; $p<0.005$).No statistically significant differences were found in the biopsy specimens for *Clostridia, Enterococci,* as well as for *Candida* yeasts. However, statistically significant differences were demonstrated for the population of *Bifidobacteria*: 10^3 CFU/g vs. 10^6 CFU/g in NYHA III-IV vs. NYHA I-II, respectively ($p<0.05$).

Conclusion: the parietal mucin layer flora changes corresponded to the changes of the lumen flora: the higher is NYHA class, the greater is the population of gram-negative microorganisms. No changes in the population levels of gram-positive flora was demonstrated for lumen flora, while in the parietal mucin layer a decrease of *Bifidobacteria* population was reported.

2.5 Changes in the structure of the intestinal wall in CHF patients with NYHA class I-IV

Changes found in the microbial landscape of both lumen flora and parietal mucin layer flora prompted us to study the large intestine wall structure in CHF patients with NYHA class I-IV.

In Group 2, no changes were observed in the biopsy specimens of large intestine mucosa. In Group 1, hyperemic vessels and focal dense lymphoid-cellular infiltrates were reported in the lamina propria of the large intestine mucosa (Fig. 4). This pattern is consistent with marked chronic inflammation.

To confirm the lymphoid nature of the infiltrates, OLA reaction (common lymphocytic antigen) was performed (Fig. 5).

A strong positive infiltrate (derivates of white blood cell line) was observed in the lamina propria of the large intestine mucosa. Particularly, intraepithelial lymphocytes were seen clearly. CD8+ staining (Fig. 6) revealed intraepithelial CD8+-positive T-lymphocytes in the superficial layer of the large intestine mucosa.

Ki67 reaction revealed positive cells of gland epithelium (Fig. 7) in the proliferation phase. At the same time, there were solitary cells positive for Muc5 reaction (i. e. containing mucin 5) in the superficial epithelium of the large intestine mucosa.

Histopathology also revealed a large number of siderophages (Fig. 8), suggesting chronic congestion in the large intestine vessels. We considered these changes to be a sign of the chronic heart failure.

Conclusion: the CHF patients with NYHA class III-IV showed signs of marked chronic inflammation in the large intestine mucosa, along with tissue edema and venous congestion. The severity of these changes increased with higher NYHA class and the severity of CHF decompensation symptoms.

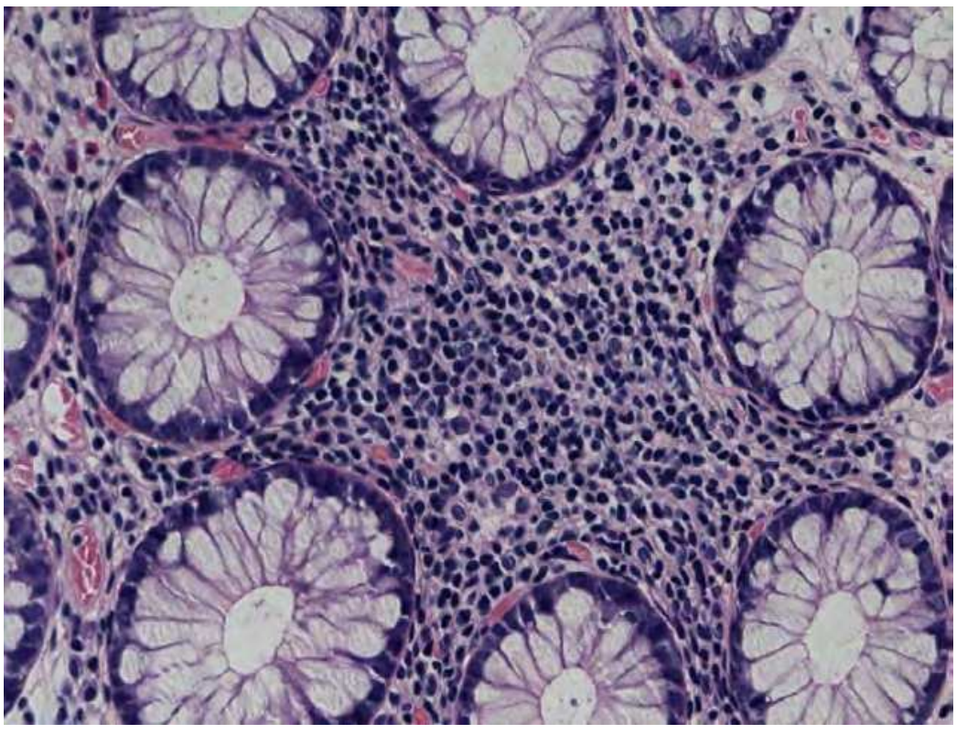


Fig. 4. Biopsy sample of the large intestine mucosa. Hematoxylin-eosin stain.

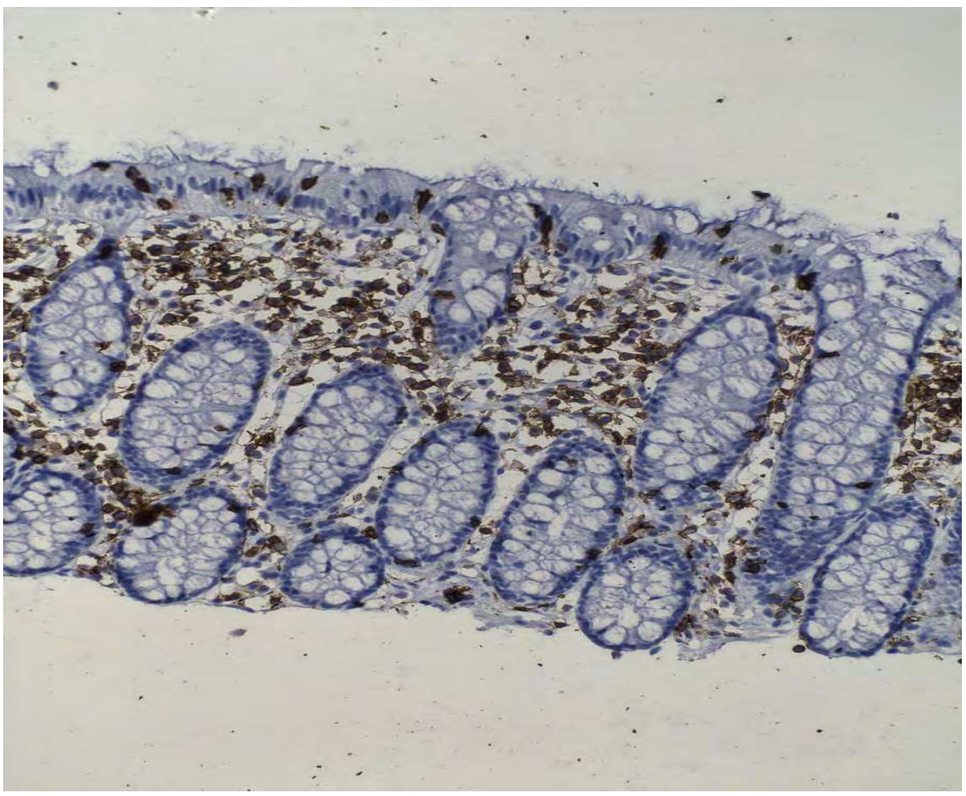


Fig. 5. Biopsy sample of the large intestine mucosa. OLA reaction.

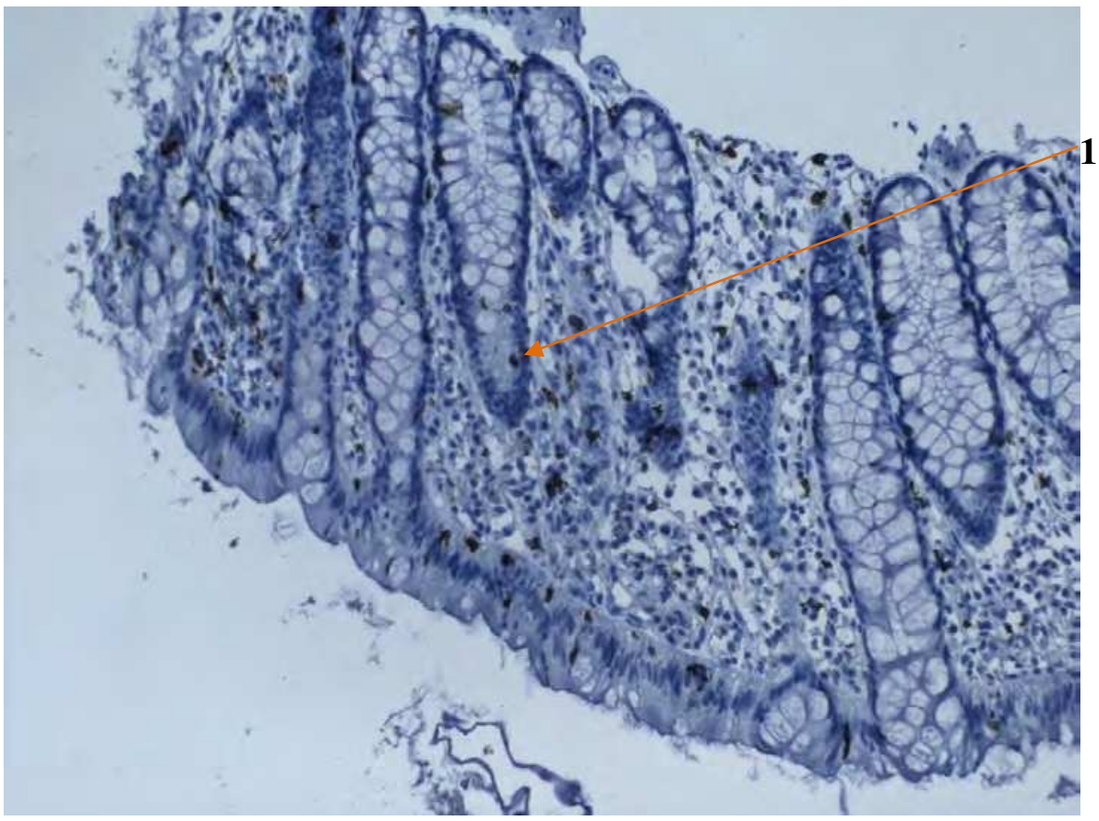


Fig. 6. Biopsy sample of the large intestine mucosa, CD8+ lymphocyte stain. 1 – CD8+ lymphocytes.

2.6 Endotoxin level assessment

Plasma levels of the endotoxin were 1.2±0.03 EU/L in NYHA class III-IV subjects and 0.46±0.01 EU/L in NYHA class I-II subjects (EU – endotoxin units). The level of the endotoxin in the control group subjects was 0.35±0.02 EU/L. Notably, plasma endotoxin levels directly correlated with the changes in population numbers of the large intestine gram-negative flora.

The CHF patients with NYHA class III-IV had levels of IL-6 at 11.5±0.3 U/L, TNF-alpha at 6.6±0.4 U/L, and CRP at 8±0.65 mg/ml. The CHF patients with NYHA class I-II had levels of IL-6 at 4.6±0.3 U/L, TNF-alpha at 3.7±0.4 U/L, and CRP at 5.5±0.29 mg/ml. The levels of these pro-inflammatory cytokines in the control group were within normal limits: : IL-6 was 2 U/L, TNF-alpha was 1.5 U/L, and CRP was 2.9 mg/ml. The identified changes prompted us to suggest two approaches to correction of these conditions:

1. Targeting the large intestine wall using different diuretic regimens, including agents with tissue activity.
2. Selective decontamination in combination with probiotics.

2.7 Use of different diuretic regimens

Figure 9 shows the study design.

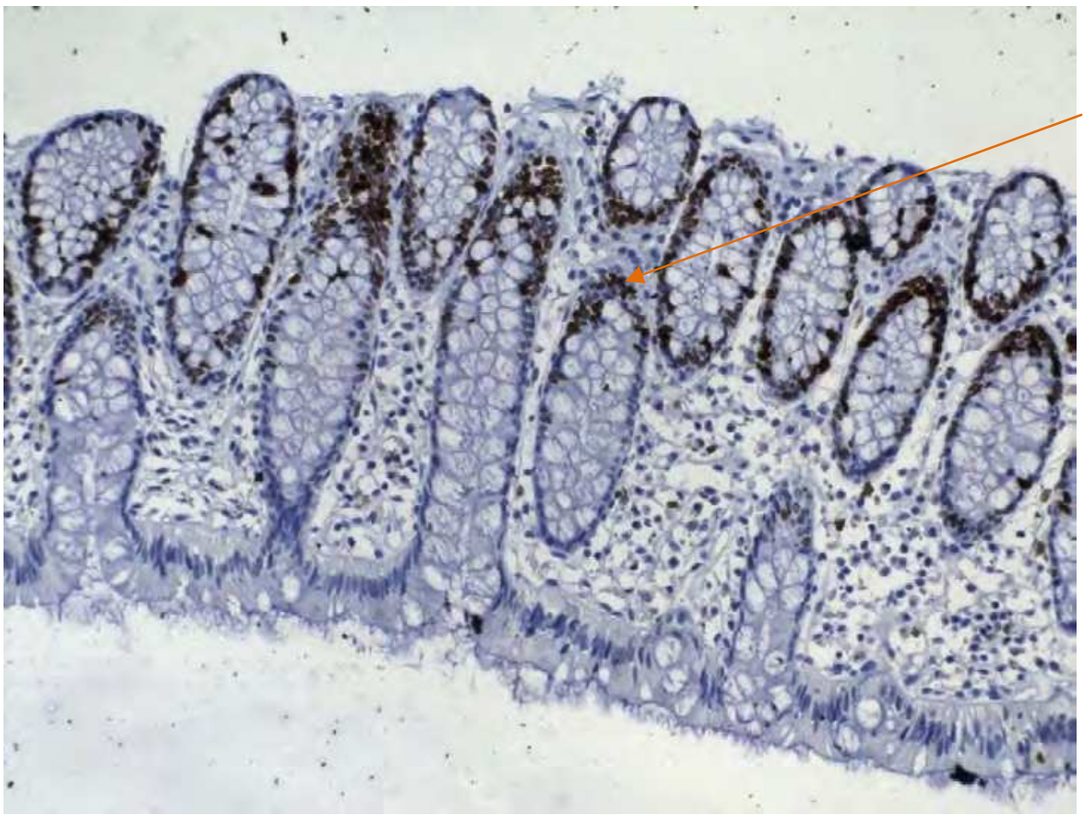


Fig. 7. Biopsy sample of the large intestine mucosa, Ki67 reaction.

1 – proliferating epithelial cells of a gland.

The study drugs were prescribed for the first 5 days after the screening visit. After that, the study drug was discontinued and patients remained on the standard-of-care therapy for the 30 days of follow-up (until compensation of their clinical status).

The following tests were performed in this study:

- body mass and the volume of excreted fluid,
- results of 6-minute test,
- Clinical Status Assessment Scale score (points),
- plasma levels of the endotoxin,
- feces flora composition and enzyme activity of the microorganisms,
- results of colonoscopy with cecum biopsy and further histopathology and histochemistry of the obtained samples.

These tests were performed on Day 1, Day 6 and Day 30 of the study.

After the study treatment period, all patients were switched to the supportive care regimen and received the standard-of-care therapy; this phase lasted for 30 days.

One patient died while on study (CHF decompensation was the cause of death).

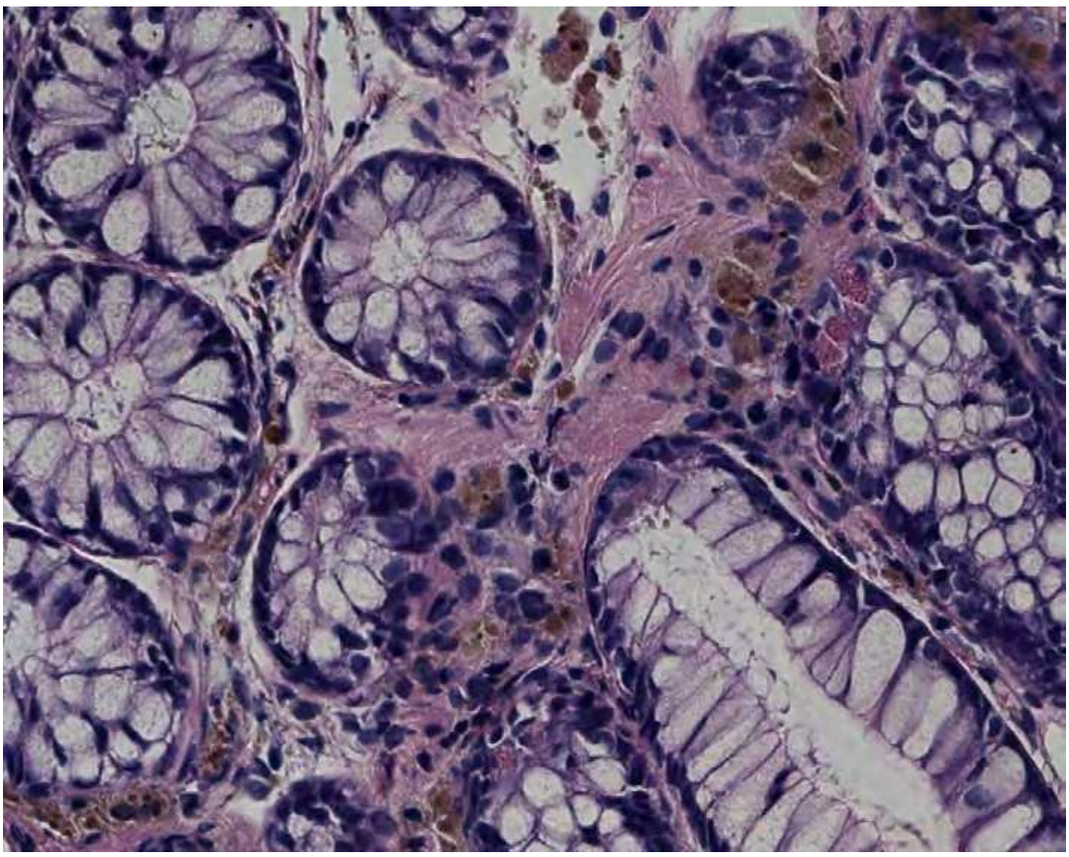


Fig. 8. Biopsy sample of the large intestine mucosa. Hematoxylin-eosin stain. 1 – siderophage.

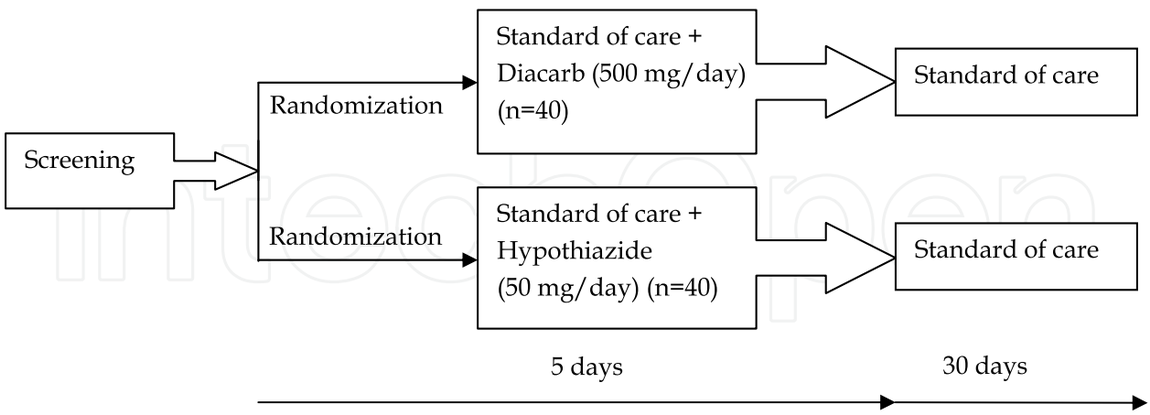


Fig. 9. Study design for the assessment of efficacy of Diacarb (acetazolamide) and Hypothiazide (hydrochlorothiazide) in comprehensive therapy of CHF patients with NYHA III-IV.

Data from 79 subjects who completed the study was therefore used for the analysis of study results.

During the study, 15 adverse reactions were reported, but none of these caused discontinuation of the study treatment.

No statistically significant differences between groups were demonstrated for the main variables.

2.8 Changes of body mass and volume of excreted urine over time

To determine efficacy of each study diuretic regimen, changes of the body mass and the volume of excreted urine were evaluated throughout the study. After the first five days, a decrease of body mass to 83±0.5 kg and 83.1±0.36 kg was demonstrated in both Group 1 and Group 2, respectively. The decrease was statistically significant against the baseline body mass of 87 kg, but the difference between groups was not statistically significant (p=0.872).

The volume of excreted urine in the study groups was the highest on the first day of treatment, comprising 2.51±0.1 L and 2.5±0.25 L for Group 1 and Group 2, respectively. This effect decreased proportionally during the following five days in both groups. Notably, no statistically significant difference was detected between groups both in terms of body mass change (p=0.99) and in terms of the volume of excreted urine.

2.9 Changes of endotoxin levels over time

Substantial decrease of endotoxin levels on the follow-up Day 21 (1.2±0.02 EU/L to 0.2±0.01 EU/L) was demonstrated in all groups. However, in the Diacarb group, this process was substantially faster: as early as on Day 5, the endotoxin levels were at 0.4±0.02 EU/L, whereas in the thiazide diuretic group the levels were at 0.78±0.01 EU/L (p=0.012). Notably, while the diuretic effects in the Hypothiazide and Diacarb groups were almost identical, the latter group demonstrated faster decrease of the endotoxin level.

This is probably due to tissue pH change caused by Diacarb, which facilitates fast dehydration of the large intestine wall and decreases its permeability for the endotoxin. To support this hypothesis, we performed histopathology and histochemistry studies using the biopsy samples from the large intestine at Day 1 and Day 5. To exclude the role of the large intestine flora, we also monitored its composition throughout the study.

2.10 Assessment of diuretic regimen effects on structural changes in the large intestine wall over time

Assessment of the histological and histochemical patterns in the large intestine mucosa during Diacarb or Hypothiazide treatment demonstrated the following changes.In the thiazide group (Fig. 10 and Fig. 11), there were signs of reduced mucosal edema on treatment (Day 6); however, a rise of local inflammatory reaction was also evident (lymphoplasmocytic infiltration, increased number of segmented WBCs, predominantly eosinophils).This may be a relative effect caused by reduction of edema and shortening of intercellular distances rather than an absolute growth of the inflammatory infiltration.

A decrease of edema on Day 6 was also seen in the Diacarb group (Fig. 12, Fig. 13), but, in addition to that, the level of local inflammation decreased (the infiltration by lymphoid cells is consistent with low-intensity chronic inflammation).

Most probably, the difference between Diacarb and Hypothiazide in their effects on large intestine wall edema causes different permeability of the wall for the endotoxin, which results in different kinetics of blood endotoxin level reduction in the CHF patients with NYHA class III-IV. These findings may result from changes in pH of the intestinal wall, better microcirculation, and consequent decrease of infiltration. Another possibility is that Diacarb, a carbonic anhydrase inhibitor, blocks alpha-carbonic anhydrase of gram-negative bacteria, depressing their pathogenic effect on the intestinal wall.

2.11 Assessment of effects of diuretic regimens on changes of flora over time

No statistically significant changes in concentration of gram-negative bacteria both in feces and in biopsy material were demonstrated, regardless of the treatment regimen.

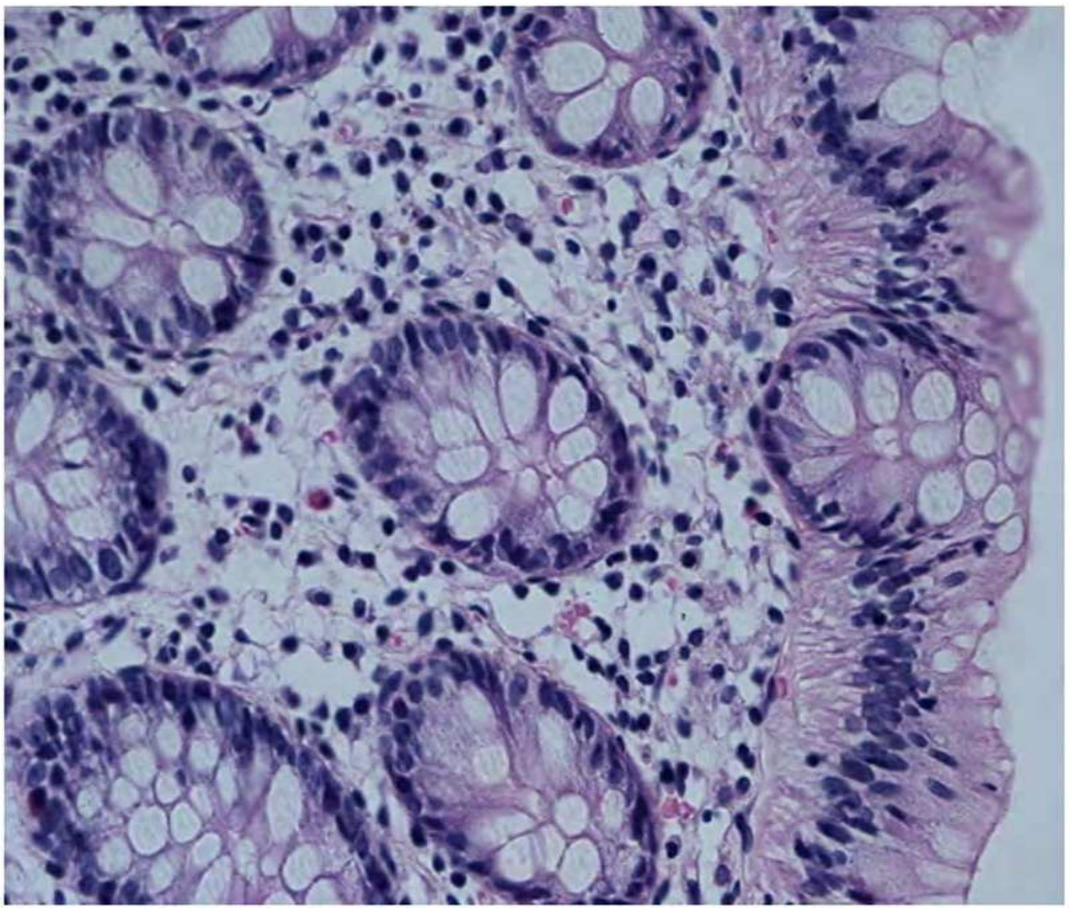


Fig. 10. Magnification x 400 (good), hematoxylin and eosin stain.Before treatment, "fuzzy" lymphoid-cellular infiltration and solitary eosinophils were noted in the *lamina propria* of the large intestine mucosa.

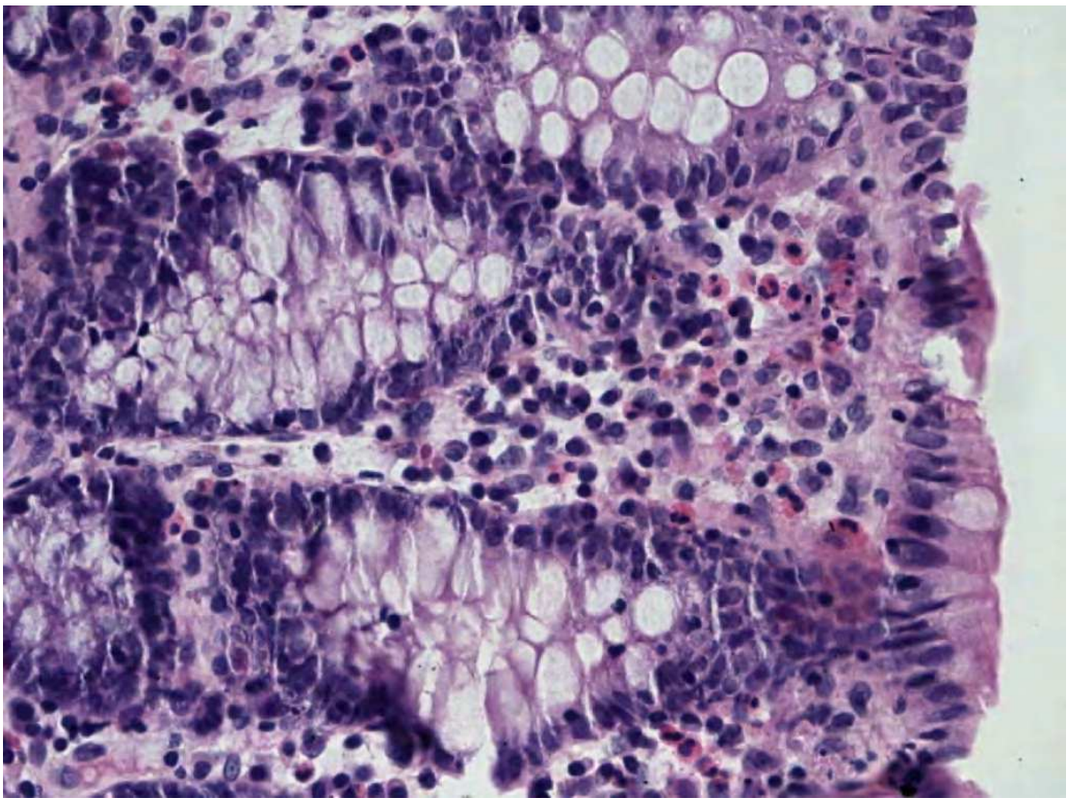


Fig. 11. Magnification x 400 (good), hematoxylin and eosin stain.Day 6 of the treatment period.Lymphoplasmocytic infiltration increases. The population of segmented WBCs, particularly eosinophils, increases significantly.

These results demonstrate that plasma endotoxin levels in CHF patients with NYHA class III-IV are affected not only by the gram-negative flora population in the intestine, but also by the severity of edema, and therefore by the degree of decompensation of patient's clinical status.The endotoxin levels in this case are probably affected by the increase of the intestinal wall permeability for the endotoxin caused by the edema.

As patient's status improves towards compensation, the endotoxin levels decrease to normal values. This process is faster with the use of Diacarb compared to the use of Hypothiazide. However, this is not accountable to their diuretic effects, because there were no statistically significant differences in the changes of body mass and the volume of excreted urine between Diacarb and Hypothiazide.It is likely that Diacarb improves microcirculation by changing tissue pH, and causes not only improvement of renal urine filtration, but also faster shrinking of tissues, particularly shrinking of the intestinal wall, which decreases its permeability for the endotoxin.

Differences in the inflammatory infiltrate intensity between Hypothiazide and Diacarb groups were discovered (in addition to decreased edema, Diacarb reduces the inflammation in the large intestine mucosa). This effect is probably accountable to improved microcirculation in the intestinal wall in the Diacarb group, as well as carbonic anhydrase inhibition produced by Diacarb leading to block of alpha-carbonic anhydrase of gram-negative bacteria, which reduces their pathogenic effect on the intestinal wall.

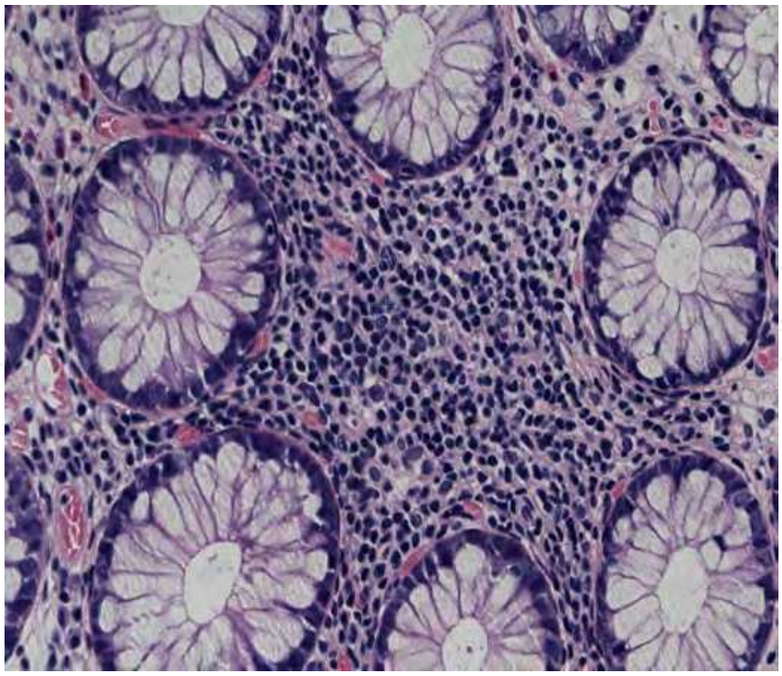


Fig. 12. Magnification x 400 (good), hematoxylin and eosin stain.Before treatment, there were hyperemic vessels and focal dense lymphoid-cellular infiltration in the *lamina propria* of the large intestine. This pattern is typical for strong chronic inflammation.

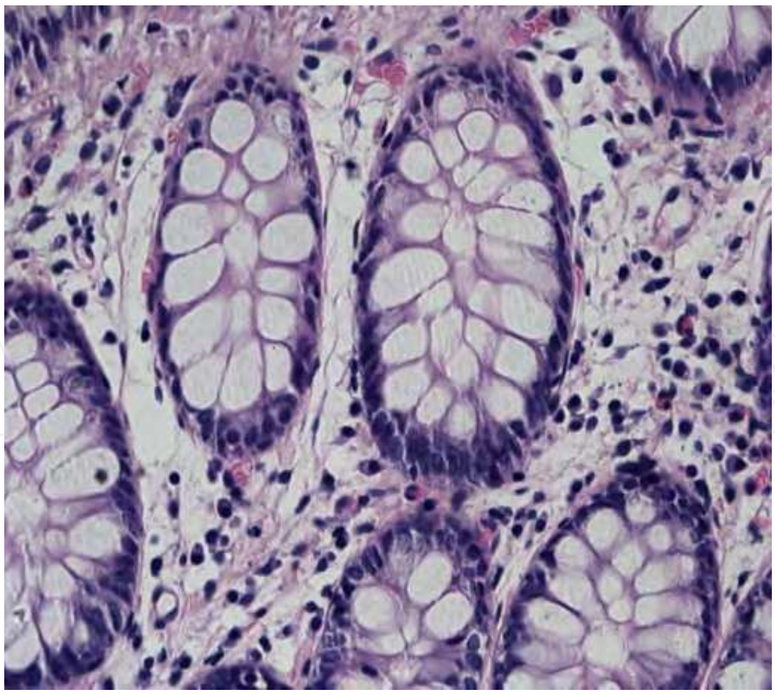


Fig. 13. Magnification x 400 (good), hematoxylin and eosin stain. Day 6 of the treatment period."Fuzzy" lymphoid-cellular infiltration is seen in the *lamina propria* of the large intestine mucosa, which is consistent with the pattern of weak chronic inflammation.

Conclusion: administration of Diacarb facilitates faster decrease of plasma endotoxin levels, which allows for faster compensation of patient's clinical status.

2.12 Use of selective decontamination alone vs. selective decontamination in combination with probiotics in comprehensive therapy of NYHA class III-IV CHF

All patients included in this phase of the study received standard-of-care therapy, including:

- ACE inhibitors/ angiotensin receptor blockers (mean daily dose of 10 mg/160 mg);
- beta-blockers (prescribed from Day 5 after the start of the therapy, dose titration from the minimum therapeutic dose, mean daily dose was 50 mg of metoprolol per day);
- digoxin 0.00025 g per day (in case of atrial fibrillation with tachycardia or LVEF below 25 %);
- aspirin 125 mg/day (secondary prophylaxis method);
- Cordarone 200 mg/day (in case of ventricular disturbances with risk of high Lown grades);
- loop diuretics (Lasix) with mean daily dose of 70 mg/day.

The following drugs were chosen for the study:

- antibacterial fluoroquinolone: ciprofloxacin with daily dose of 1000 mg;
- probiotic: Primadophilus Bifidus, 1 capsule per day.

At the study start, all patients received a 5-day selective decontamination with oral ciprofloxacin 1000 mg/day.After that, patients were randomized into two groups:

- Group 1 (n=45) received standard-of-care;
- Group 2 (n=45) received the probiotic – Primadophilus Bifidus, 1 capsule per day for 14 days.

After the completion of probiotic treatment, both groups received standard-of-care.

The following study variables were evaluated on Day 1, Day 5, and Day 21:

- results of 6-minute test,
- Clinical Status Assessment Scale score (points),
- plasma levels of the endotoxin,
- feces flora composition and enzyme activity of the microorganisms.

In this phase, 2 deaths occurred in the group receiving selective decontamination alone.In the Primadophilus Bifidus group, one patient experienced adverse effects leading to patient's decision to discontinue the drug.

Data from 87 subjects who completed the study was therefore used for the analysis of study results.

Ten cases of adverse reactions were reported during the study. Only one of these cases caused the patient to stop the drug.

The resulting groups had comparable basic characteristics.

2.13 Changes of the large intestine flora over time with the use of selective decontamination alone or selective decontamination combined with the probiotic

By Day 5 of the study, a statistically significant decrease was demonstrated in Group 1 for total population of both gram-negative microorganisms (baseline value was $10^{12\pm0.1}$ CFU/g, the value after the selective decontamination was $10^{6\pm0.4}$ CFU/g; p=0.000) and gram-positive microorganisms (baseline value was $10^{6\pm0.56}$ CFU/g, the value after the selective decontamination was $10^{4\pm0.32}$ CFU/g; p=0.000)However, on Day 21 study visit, gram-negative and gram-positive populations returned to their baseline levels ($10^{11.9}$ CFU/g and $10^{6.1}$ CFU/g, respectively; p=0.000). These results support literature reports of low efficacy of the selective decontamination used alone.

In the group where probiotics were prescribed after the course of selective decontamination, Day 5 populations decreased similarly to Group 1, both for gram-negative (baseline $10^{12.05\pm0.6}$ CFU/g, post-decontamination $10^{6.3\pm0.4}$ CFU/g; p=0.000) and gram-positive (baseline $10^{5.2\pm0.5}$ CFU/g, post-decontamination $10^{4.2\pm0.2}$ CFU/g; p=0.000) microorganisms.However, after Primadophilus Bifidus administration for 14 days, gram-positive flora population grew to $10^{8.02\pm0.1}$ CFU/g, and an insignificant growth of gram-negative flora to $10^{7.27\pm0.1}$ CFU/g was demonstrated, which is consistent with normal values for gram-negative population in the large intestine.

Conclusion: administration of probiotics after a course of selective decontamination normalizes large intestine flora levels, whereas decontamination alone leads to reduction of microbial populations for a short term only.

2.14 Changes of the endotoxin level over time

In Group 1, Day 5 endotoxin levels decreased from baseline significantly (baseline: 1.2±0.9 EU/L, Day 5: 0.55±0.06 EU/L; p=0.000), which corresponded to the reduction of gram-negative population in the large intestine. However, at Day 21, as the gram-negative population in the large intestine grew, the plasma endotoxin levels returned to their baseline values (1.18±0.05 EU/L); on Day 30, the endotoxin concentration remained high (1.21±0.045 EU/L).

In Group 2, after the selective decontamination was completed and the gram-negative population in the large intestine reduced, the plasma endotoxin levels also declined (baseline: 1.24±0.01 EU/L, Day 5: 0.67±0.03 EU/L, p=0.000). A trend towards decline of plasma endotoxin levels and achievement of normal values was demonstrated subsequently (Day 21: 0.56±0.02 EU/L, Day 30: 0.26±0.08 EU/L). These results can be explained by the reduction of the gram-negative populations in the large intestine due to administration of the selective decontamination followed by probiotic.

2.15 Assessment of changes in plasma pro-inflammatory cytokine levels over time

With the use of the selective decontamination alone, the decrease of the following variables on Day 5 was demonstrated: IL-6 to 4.1±0.03 U/L (baseline 4.9±0.01 U/L), TNF-alpha to 5±0.09 U/L (baseline 5.7±0.04 U/L), and CRP to 4 mg/ml (baseline 8.6 mg/ml).However, as early as on Day 12, these cytokine variables were shown to return to their baseline levels,

which persisted till Day 30. This demonstrates poor efficacy of the selective decontamination alone for the system inflammation marker endpoints.

When the selective decontamination was used in combination with the probiotic, decrease in the population of enterobacteria in the large intestine and decrease of plasma endotoxin levels were reported.However, while levels of the pro-inflammatory cytokines decreased by Day 5 (CRP: 4.2±0.1 mg/ml on Day 5 vs. baseline 7.82±0.05 mg/L; IL-6: 3.9±0.05 U/L on Day 5 vs. baseline 5±0.01 U/L; TNF-alpha: 5.01±0.02 U/L on Day 5 vs. baseline 5.8±0.02 U/L), but on Day 21 they already rebounded above the baseline levels, and on Day 30 there was a trend towards further growth of their levels.

This is probably accountable to the decrease of gram-negative flora population in the large intestine due to the antibacterial treatment, and consequent decline of plasma levels of the endotoxin.Normal population numbers of the gram-negative flora are further maintained by the administration of the probiotic. However, the probiotic contains gram-positive microorganisms, which bind to the Toll-like receptors, initiating the synthesis of pro-inflammatory cytokines in the large intestine enterocytes, leading to further exacerbation of the systemic inflammation.

These results demonstrate lack of efficacy for the selective decontamination used alone.When the selective decontamination was combined with the probiotic, normalization of the intestinal flora and plasma endotoxin levels was reported. However, a significant growth of the pro-inflammatory cytokine levels occurs with this regimen, which affects patient's status and is likely to require additional correction.

These data demonstrated that comprehensive therapy for CHF combined with the selective decontamination alone (i. e. without probiotic) caused to a short-term decline of gram-negative flora population, while the population numbers of gram-positive flora remained almost unaffected. A short-term decline in plasma levels of the endotoxin and pro-inflammatory cytokines was also reported. However, as early as one week after the discontinuation of the antibacterial treatment, gram-negative flora population numbers returned to their baseline levels, accompanied by the increase in plasma levels of the endotoxin and the pro-inflammatory cytokines. A potential explanation for this pattern is that CHF patients with NYHA class III-IV develop significant restructuring of their large intestine walls, providing favorable conditions for domination of gram-negative flora.Isolated use of the selective decontamination, neither supported by any agents that repair large intestine wall structure, nor combined by any probiotics, fails to provide stable, long-term changes in the large intestine flora.

However, administration of probiotics added to the antibacterial treatment demonstrated persistent effect: normalization of gram-negative flora levels and plasma endotoxin levels. Notably, with the use of the probiotic, blood CRP levels increased, which is probably accountable to the presence of gram-positive flora in the probiotic, prompting the host to produce more antibodies. Unfortunately, the CRP levels were not followed for a longer period of time, and the time needed for the CRP to reach normal levels remained unknown.It can only be assumed that this period should not take too much time, because the subjects were exposed to the probiotic for 2 weeks only. However, if a long-term, persistent growth of the CRP levels in blood do occur, decompensated CHF patients with NYHA class III-IV might benefit from administration of statins.

3. Clinical significance of adipose tissue changes over time in patients with chronic heart failure of ischemic origin. Treatment options

3.1 Introduction

Syndrome of cardiac cachexia is one of the most severe complications of chronic heart failure. Among the latest advancements in the field of immunology is the concept of cytokine activation system and its role in the pathogenesis of chronic heart failure and development of cardiac cachexia. Currently, two main classes of cytokines are known to participate in the development of heart failure:vasoconstrictive cytokines (endothelin-1 and big endothelin) and vasodepressive cytokines (TNF-α, IL-1, IL-6, IL-8). Patients with signs of cardiac cachexia are known to have higher levels of inflammation markers than patients with normal body mass (Francis, 1998; Monteiro, 2007). Notably, adipose tissue is one of the sources of cytokines.In addition to leptin and adiponectin, adipose tissue was demonstrated to participate in production of TNF-α and IL-6 (Moses, 2004; Nagaya, 2001; Springer, 2010).

We assumed that one of the methods to decrease the activities of pro-inflammatory cytokines could be the increase of dry body mass (body muscle mass, body fat mass) (Dostalova et al., 2003). Therefore, one of the options to correct the levels of inflammatory markers in this category of patients could be nutritional support.

From our perspective, there are two possible approaches to correct the systemic inflammation:development of methods that can increase the mass of the adipose tissue and methods that have direct effect on the synthesis of pro-inflammatory cytokines.

3.2 Assessment of body composition, levels of leptin, adiponectin, and pro-inflammatory cytokines in patients with different NYHA classes of CHF

To study these variables, three consecutive groups were enrolled in the first part of the study: Group 1 included chronic heart failure patients with NYHA class I-II, Group 2 included chronic heart failure patients with NYHA class III-IV (subgroup A: without cachexia; subgroup B: with cachexia), Group 3 was a control group.Patient screening was performed in the population of patients with history of CHF of ischemic origin with NYHA class I-IV for more than 6 months, older than 40 years of age, admitted to a general internal medicine department or a cardiology department (n=197).The control group included outpatients of the Consultation and Diagnostics Polyclinic (n=52).

Clinical characteristics of the patients:

Age:these three groups were comparable in terms of patients' age.

From the results of analysis of associated clinical conditions, diabetes mellitus was reported in 57.1 % of CHF patients with NYHA class III-IV with cachexia and in 48.6 % without cachexia, as well as in 14 % of patients with NYHA class I-II.

Analysis of concomitant medications: CHF patients with NYHA class III-IV with/without cachexia were on ACE inhibitors or ARB in 64.2 %/63.8 %, on beta-adrenoblockers in 82.1 %/80 %, on digoxin in 75 %/76.1 %, on diuretics in 35.7 %/34.3 %, and on Cordarone in 28.6 %/19.5 % of cases, respectively.For NYHA class I-II patients, the corresponding variables were: on ACE inhibitors/ARB 51.6 %, on beta-adrenoblockers 59.4 %, on digoxin 9.3 %, on diuretics 45.3 %, on Cordarone 3.1 %.

3.3 Comparison of methods used to evaluate body composition in patients with CHF of different NYHA class and the control group patients

Two methods were used to study body composition: caliper measurement and bioimpedance analysis.Comparison of these methods in all three groups did not reveal any statistically significant differences between the values obtained using caliper measurements and bioimpedance analyzer.However, the method of caliper measurements is subjective and is not suitable for patients with decompensated heart failure (i. e. with severe edema syndrome).Moreover, unlike caliper measurements, bioimpedance analyzer of body composition allows to estimate not only the body fat mass, but also total fluid content, which is important for the assessment of the body composition in patients with decompensated chronic heart failure.

Comparison of Group 1 vs. Group 2 revealed statistically significant differences in the adipose tissue mass, which was 29.7±2.2 kg in Group 1 and 22.2±2.1 kg in Group 2 ($p<0.001$), as well as in the lean body mass, which was 54.8±6.9 kg in Group 1 and 59.7±6.1 kg ($p<0.001$) in Group 2. This was associated with changes in total fluid content, which was 38.2±3.1 kg in Group 1 vs. 48.8±3.3 kg in patients with NYHA class III-IV ($p<0.001$).BMI did not differ significantly between groups.

Comparison of the study results between Group 2 (NYHA III-IV) and the control group revealed changes similar to those between Group 1 and Group 2.

Comparison of Group 1 (NYHA I-II) vs. the control group revealed statistically significant differences in the body fat mass, which was 29.7±2.2 kg in Group 1 vs. 32.6±2.4 kg in Group 3 ($p<0.001$), and statistically significant differences in total fluid content, which was 38.2±3.1 kg in Group 1 vs. 25.6±2.9 kg in Group 3 ($p<0.001$).

In patients with cachexia, a significantly lower LBM of 55.2±4.9 kg and body fat mass of 15.65±1.8 kg were reported. Notably, the total fluid content levels in these patients was greater than levels of this variable in patients without cachexia, but the differences of this variable were not statistically significant.

Conclusion: the higher is NYHA class of CHF, the stronger are changes of body composition; with higher NYHA class, adipose tissue mass declines, total fluid content grows.These changes were stronger in patients with cachexia.

3.4 Levels of leptin, adiponectin, and pro-inflammatory cytokines in patients with different NYHA classes and in the control group

The patients with NYHA III-IV were found to have adiponectin levels at 18.6±4.9 μg/mL, leptin levels at 43.8±8.3 ng/mL, IL-6 levels at 11.7±0.3 U/L, TNF-α levels at 6.6±0.2 U/L, CRP levels at 8.8±0.4 mg/ml. These values were significantly higher than values reported for patients with NYHA class I-II and for patients in the control group, who had their levels of adipokines and pro-inflammatory cytokines within normal range.

Comparison of patients with NYHA class III-IV with and without cachexia demonstrated the following differences: leptin levels were significantly higher in patients without cachexia (47.9±4.2 ng/mL), while levels of adiponectin, IL-6, TNF-α, and CRP were significantly higher in patients with cachexia.

Conclusion: the higher is NYHA class of CHF, the stronger is the intensity of chronic inflammation. While this may be an effect of growing intoxication in patients with advanced stages of CHF, this could also be associated with the role of the adipose tissue in production of pro-inflammatory cytokines and biologic agents that stimulate cytokine production. Higher classes of NYHA are associated with lower adipose tissue mass, which leads to increase of adiponectin plasma levels.However, the levels of leptin in patients with NYHA class III-IV are also high, which may be accountable to big dimensions of adipocytes. Patients with cachexia have significantly lower levels of leptin when compared to patients without cachexia; this is probably associated with shrinking of the lipid droplet in the adipocyte.

3.5 Evaluation of visceral and subcutaneous tissue structure in patients with different CHF classes

To study this variable, autopsies of deceased patients (with NYHA class I-IV chronic heart failure diagnosed before their death) were performed. Patients were allocated into two groups: Group 1: before death, patients were diagnosed with NYHA I-II chronic heart failure; Group 2: before death, patients were diagnosed with NYHA III-IV chronic heart failure. Patients of Group 2 were divided in two subgroups: patients with cachexia and patients without cachexia. All patients were admitted to GKB no. 4 (City Clinical Hospital no. 4) before death. For NYHA I-II patients, the main reasons for hospitalization were: unstable angina, acute myocardial infarction, hypertensive crisis, heart rhythm disorder, cerebrovascular accident; for NYHA III-IV patients, the main reasons for hospitalization were: decompensation of CHF, acute/recurrent myocardial infarction, heart rhythm disorder, cerebrovascular accident.The postmortem assessment included measurement of the subcutaneous fat, measurement of the omentum mass, morphometric study of the subcutaneous fat, omental fat and pericardial fat.

Of 118 subjects total, 50 subjects were in the first group, 56 subjects were in the second group, and 12 subjects belonged to the third group. These three groups were comparable in terms of patients' age. The main cause of death for NYHA I-II patients was the acute myocardial infarction (46.0 %), and for NYHA II-III patients (both with cachexia and without cachexia) the main cause of death was post-infarction cardiosclerosis (58.3 %, 48.2 %, respectively). The most frequent complication leading to death in patients with NYHA class I-II CHF was acute heart failure; in patients with NYHA class III-IV the most frequent complication leading to death was CHF decompensation. Pneumonia incidence in patients with NYHA class III-IV was higher (22 %) than in NYHA I-II patients (4 %). Multiple complications were reported for 22.0 % patients with NYHA I-II, 46.4 % patients with NYHA III-IV without cachexia, and 100 % of patients with NYHA III-IV with cachexia.

During autopsy, the following investigations were performed: measurement of subcutaneous fat thickness 2 cm below the navel, measurement of omentum weight; autopsy samples of subcutaneous fat, omental fat, and pericardial fat at the apex of the heart were collected.

There were no statistically significant differences between patients with NYHA I-II and NYHA III-IV without cachexia in the thickness of subcutaneous fat: it was 5.3±1.7 cm and 5.1±2.2, respectively (p=0.6).In CHF patients with NYHA III-IV with cachexia, the difference

in thickness of subcutaneous fat (2.4±1.1 cm) was statistically different from NYHA III-IV patients without cachexia ($p<0.001$).

In CHF patients with NYHA class III-IV, omentum weight was significantly lower than in patients with NYHA I-II (387±134 g vs. 521±142 g, respectively; $p<0.001$).In CHF patients with NYHA classes III and IV, the omentum weight was 164±87 g, which is significantly lower than in patients without cachexia ($p<0.001$).

The morphometric analysis of the samples showed the following changes.In the subcutaneous fat, lymphocytic infiltration was the strongest in patients with NYHA class III-IV with cachexia (average 12.4±4.7 %); in patients with NYHA class I-II this variable was 3.8±2.2 % (comparison: $p<0.001$), and in NYHA III-IV patients without cachexia it was 4.3±2.4 % (comparison: $p<0.001$).No significant differences in the proportion of fibrous tissue in the subcutaneous fat was found between the groups.The percentage of fibrous tissue was 3.8±1.9 % in NYHA class I-II patients, 4.1±2.3 % in NYHA class III-IV patients without cachexia, and 4.6±2.6 % in NYHA class III-IV patients with cachexia ($p1\text{-}2=0.469$, $p1\text{-}3=0.229$, $p2\text{-}3=0.506$). See Fig. 14, 15, 16.

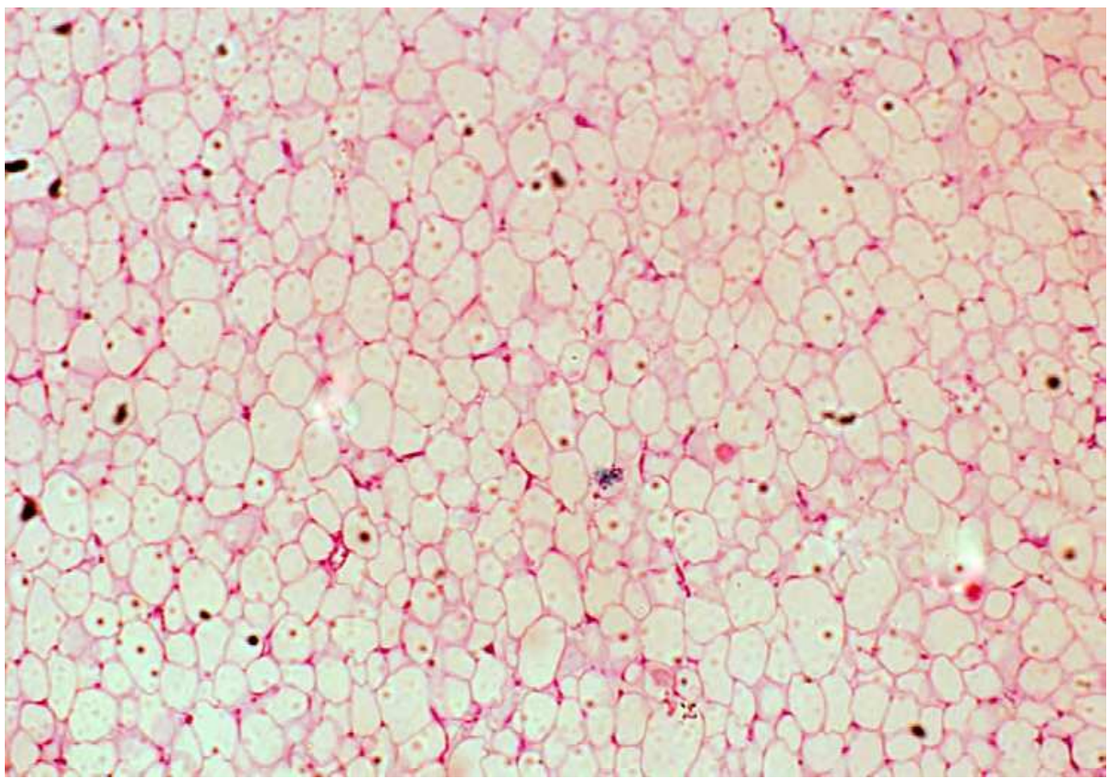


Fig. 14. Subcutaneous fat in a CHF patient with NYHA class I. Romanowsky-Giemsa stain.

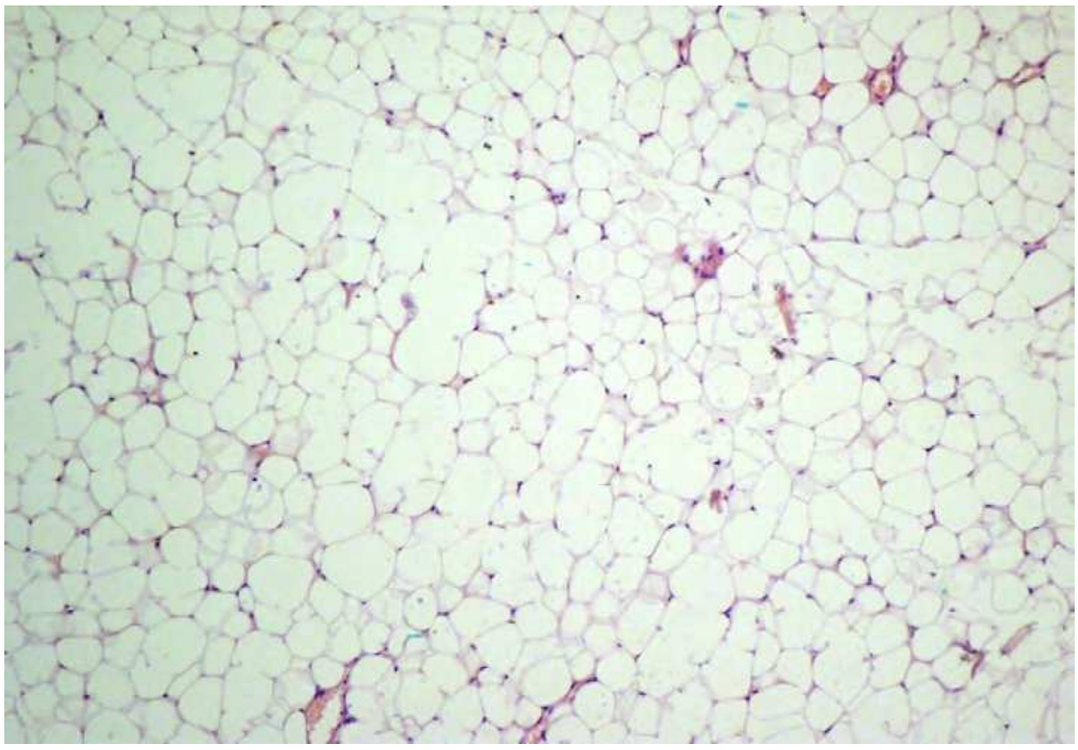


Fig. 15. Subcutaneous fat in a CHF patient with NYHA class III.

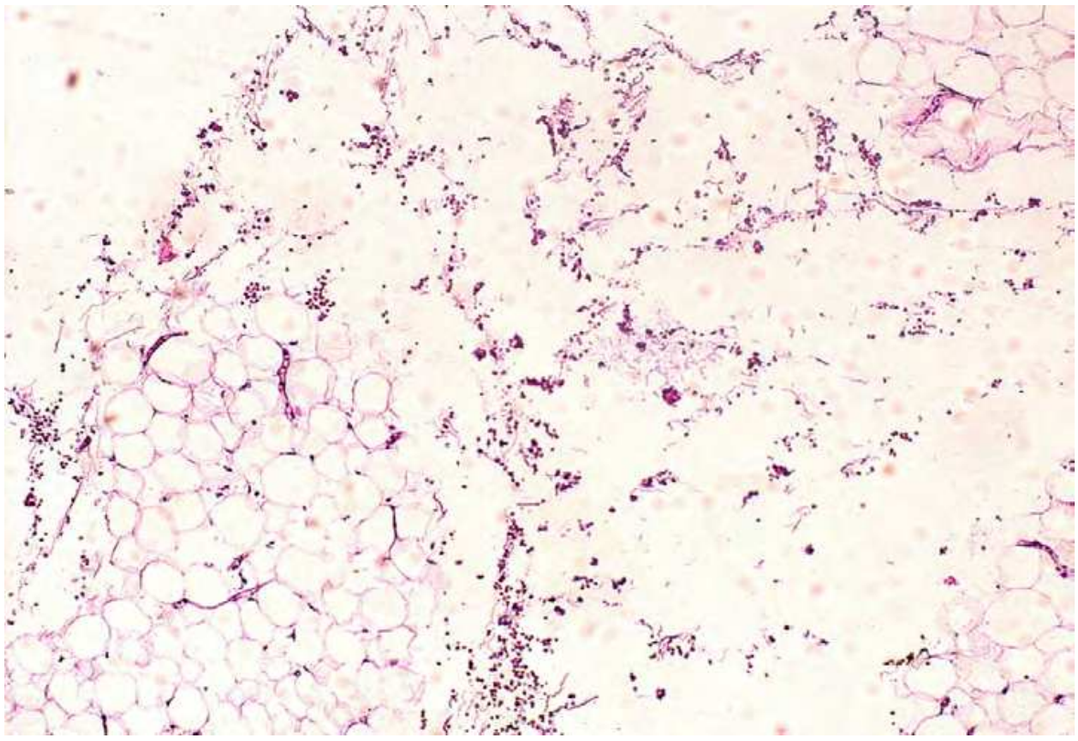


Fig. 16. Subcutaneous fat in a CHF patient with NYHA class IV with cachexia. Romanowsky-Giemsa stain.

Analysis of the visceral fat (omentum, pericardial fat) showed the strongest lymphocytic infiltration in subjects with NYHA class III-IV, both with cachexia and without cachexia. This variable was 53.4±7.8 % in the omentum and 49.7±8.4 % in the pericardium in patients with cachexia, and 42.1±6.7 % in the omentum and 42.6±8.8 % in the pericardium in patients without cachexia (compare to patients with NYHA I-II, who had the lymphocytic infiltration of 5.1±2.3 % in the omentum ($p1<0.001$, $p2<0.001$) and 4.9±2.6 % in the pericardium ($p1<0.001$, $p2<0.001$)).The amount of fibrous tissue in NYHA class III-IV patients without cachexia was higher than in the patients with NYHA class I-II: 15.2±4.9 % vs. 3.1±1.2 % in the omental adipose tissue ($p<0.001$), 14.8±5.4 % vs. 3.2±0.9 % in the adipose tissue of the pericardium ($p<0.001$), respectively.In NYHA III-IV patients with cachexia, the amount of fibrous tissue was significantly higher than in NYHA III-IV patients without cachexia: 24.8±3.7 % in the omentum ($p<0.001$) and 24.3±3.2 % in the pericardium ($p<0.001$).See figures 17-22.

Mean diameter of adipocytes was measured, with the following results:In CHF patients with NYHA class I-II, the diameter of adipocytes was 38.6±12.2 μm in the subcutaneous fat samples, 44.2±16.1 μm in the omentum, 42.3±11.4 μm in the pericardial fat. In CHF patients with NYHA class III-IV without cachexia, the diameter of adipocytes was 42.7±14.2 μm in the subcutaneous fat samples ($p=0.116$), 56.4±13.9 μm in the omentum ($p<0.001$), 52.2±11.3 μm in the pericardial fat ($p<0.001$). In NYHA class III-IV patients with cachexia, the diameter of adipocytes was 28.2±11.5 μm in the subcutaneous fat samples ($p=0.01$), 32.8±14.3 μm in the omentum ($p=0.028$), 30.3±12.4 μm in the pericardial fat ($p<0.001$).

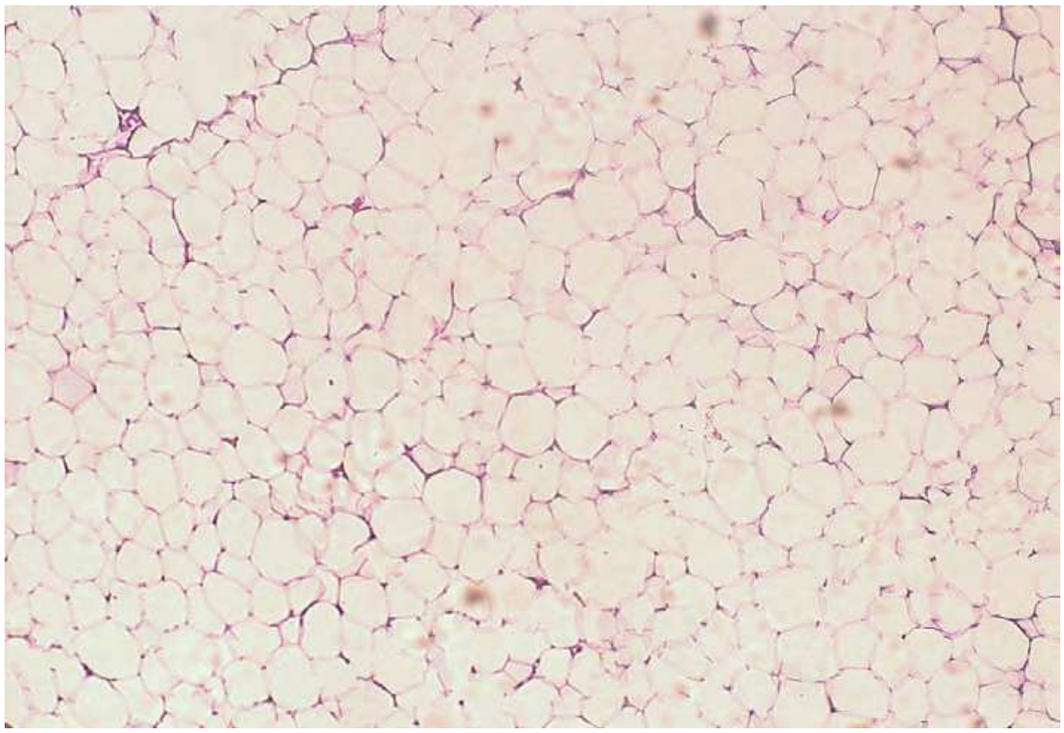


Fig. 17. Visceral adipose tissue (pericardial fat) in a CHF patient with NYHA class I. Romanowsky-Giemsa stain.

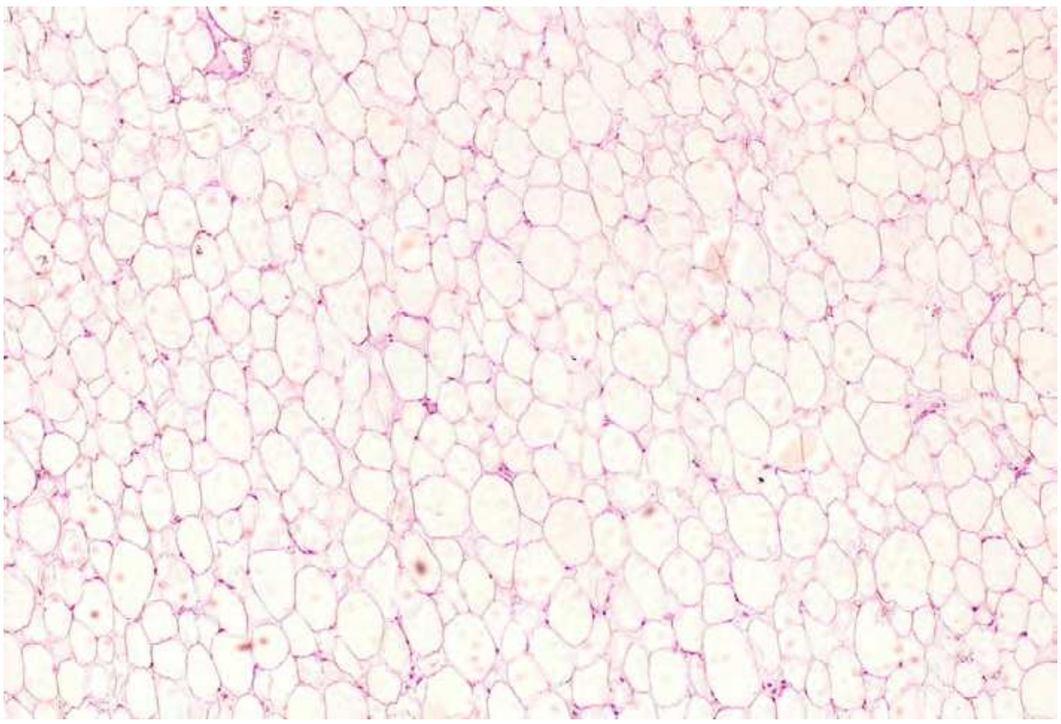


Fig. 18. Visceral adipose tissue (omentum) in a CHF patient with NYHA class I. Romanowsky-Giemsa stain.

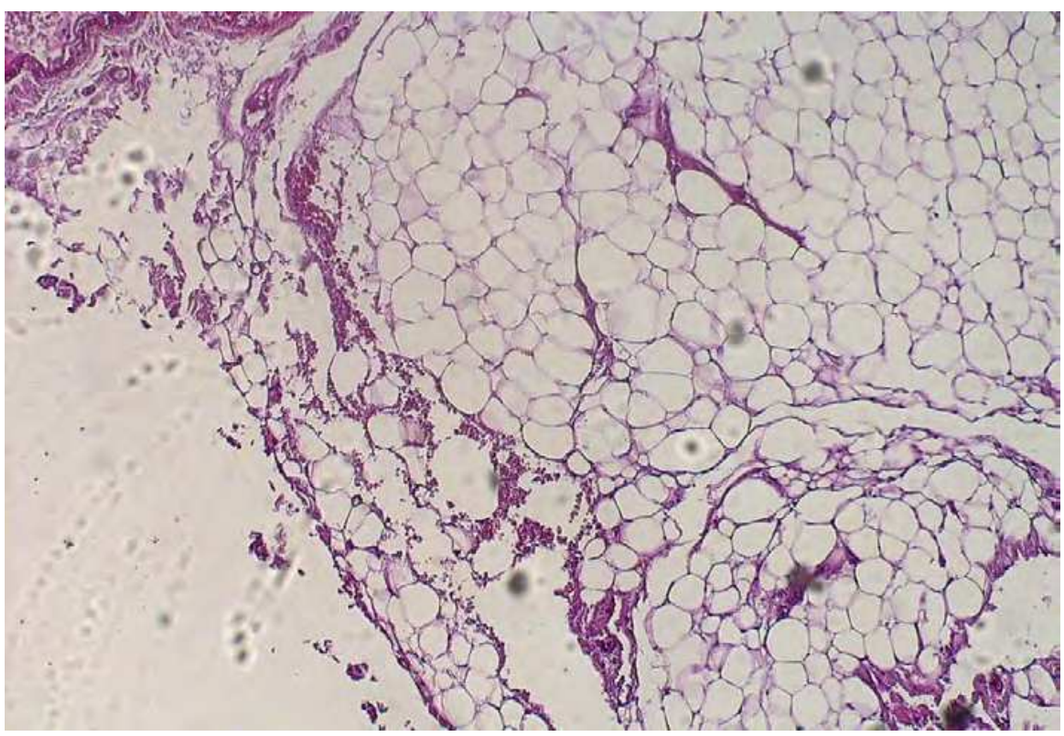


Fig. 19. Visceral adipose tissue (pericardial fat) in a CHF patient with NYHA class III.Romanowsky-Giemsa stain.

Fig. 20. Visceral adipose tissue (omentum) in a NYHA class III patient without cachexia. Van Gieson's stain.

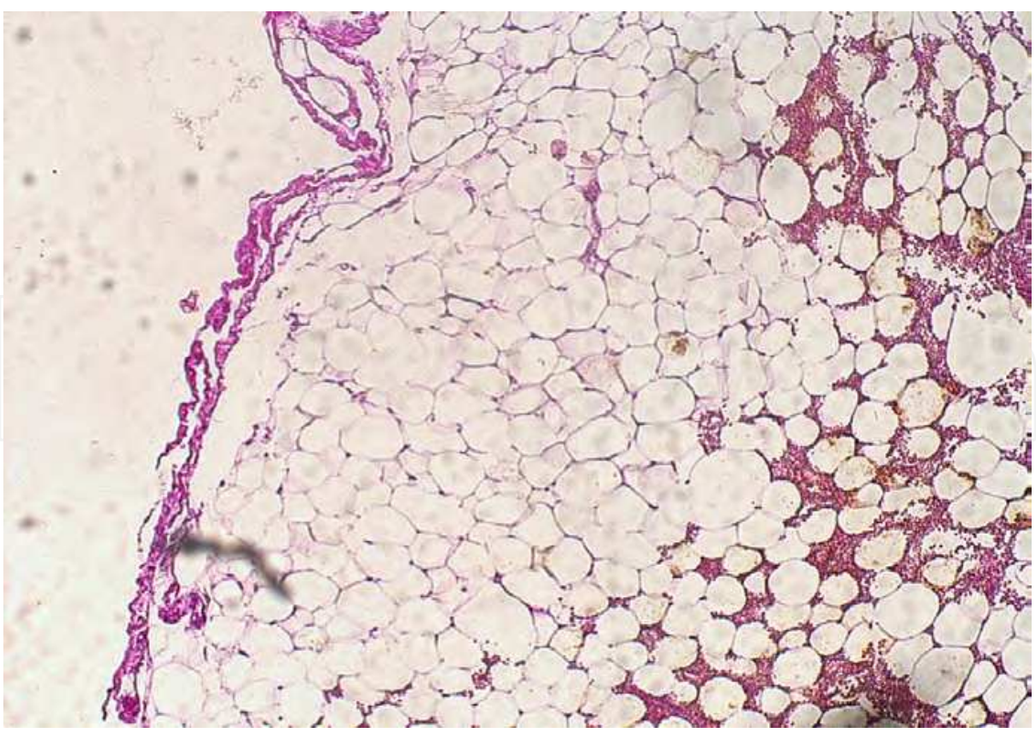


Fig. 21. Visceral adipose tissue (pericardial fat) in a CHF patient with NYHA class IV with cachexia.Romanowsky-Giemsa stain.

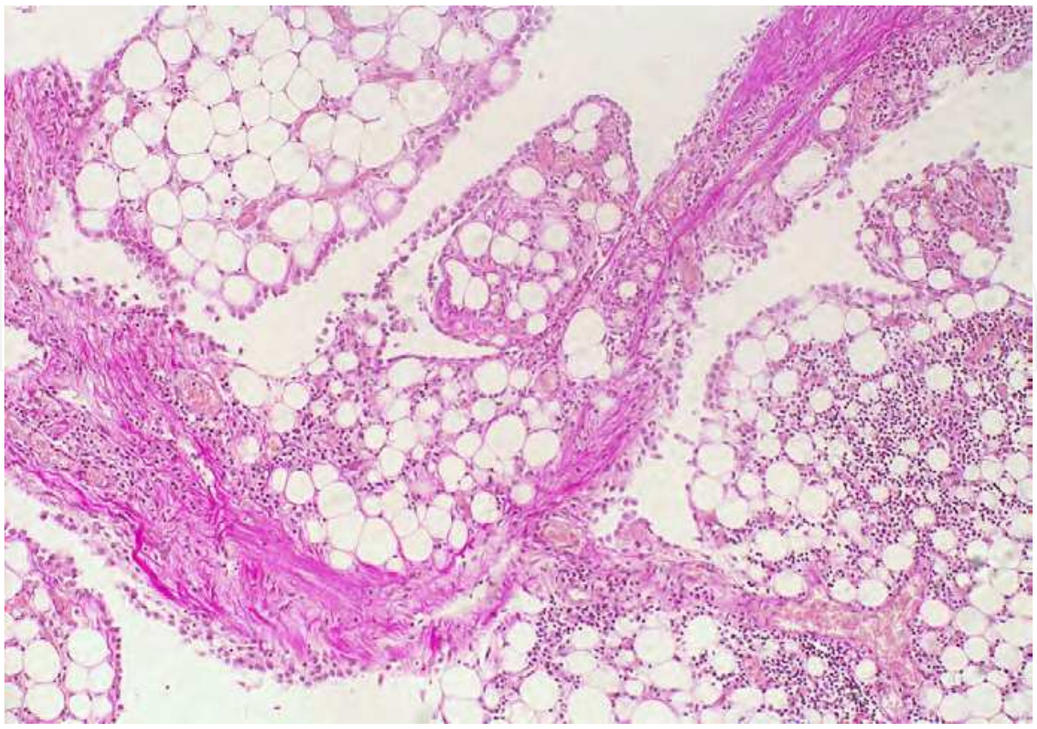


Fig. 22. Visceral adipose tissue (omentum) in a NYHA class IV patient with cachexia. Van Gieson's stain.

Therefore, the higher is NYHA class, the lower are the subcutaneous fat and the omentum weight. The higher is NYHA class, the more intensive is the chronic inflammation, manifested by the lymphocytic infiltration and the content of fibrous tissue, especially in the visceral fat.These changes are stronger in NYHA III-IV patients with cachexia.

In patients with NYHA III-IV and cachexia, a decrease in the content of adipose tissue was demonstrated to be the result of adipocyte shrinking, as well as substitution of the adipose tissue by fibrous tissue.

Currently, there is no solution for the problem of how to increase the body muscle mass and body fat mass in patients with cachexia, particularly with cardiac cachexia. Unfortunately, all attempts to use anabolic steroids in this patient population have been unsuccessful.We assumed that using nutritional support in this patient population would allow to change the nutritional status in addition to reducing the malabsorption syndrome. Moreover, nutritional mixes that have both local and systemic anti-inflammatory effect are currently available. Administration of these mixes might help fighting the inflammation observed in the large intestine of the CHF patients with higher classes of NYHA, as well as reducing their blood levels of pro-inflammatory cytokines.

3.6 Comparison of efficacy of Modulen vs. Peptamen added to standard-of-care therapy in CHF patients with NYHA III-IV

Screening was performed in a consecutive population of patients hospitalized to general internal medicine or cardiology departments (n=144).

Inclusion and exclusion criteria are shown in Table 5.

Inclusion criteria	Exclusion criteria
• CHF of ischemic origin • CHF history for more than 12 months • Age > 40 years old • Patient's consent to participation in the study	• Acute or chronic infectious diseases • Cancer within last 5 years • Severe impairment of liver or kidneys (AST, ALT > 3 x upper limit of normal, creatinine > 250 μmol/L) • Mental disorders • Primary or secondary immunodeficiency conditions • Alcohol or substance dependence • Any conditions that can cause cachexia (at investigator's discretion) • Lack of tolerance to enteral nutrition regimen • Unable to sign the informed consent • Unable to follow the study procedures

Table 5. Inclusion and exclusion criteria.

Chronic heart failure patients with NYHA class III-IV were randomized into three groups, 40 subjects in each:

- Patients in Group 1 received Modulen (balanced nutritional mix for enteral tube feeding or oral feeding) in addition to standard-of-care therapy.
- Patients in Group 2 received Peptamen (balanced nutritional mix for enteral tube feeding or oral feeding) in addition to standard-of-care therapy.
- Patients in Group 3 received the standard-of-care therapy only, as well as the necessary amount of nutrients in a standard diet designed for cardiology patients.

All patients received standard-of-care therapy, which included:

- ACE inhibitors/ angiotensin receptor antagonists (mean daily dose of 10 mg/160 mg);
- beta-blockers (prescribed from Day 5 after the start of the therapy, dose titration from the minimum therapeutic dose, mean daily dose was 12.5 mg of carvedilol per day);
- digoxin 0.00025 g per day (in case of atrial fibrillation with tachycardia or sinus rhythm with LVEF below 25 %);
- aspirin 125 mg/day (secondary prophylaxis method);
- loop diuretics (Lasix) with mean daily dose of 60 mg/day.

The following procedures were performed for every patient:medical history; physical examination, including measurement of weight, height, waist circumference, hip circumference, wrist circumference, arm circumference; casual BP; heart rate; fasting chemistry lab blood samples; 6-minute test; echocardiography for the measurement of the ejection fraction; caliper measurements; bioimpedance analysis of body composition to measure the lean body mass (LBM), body fat mass (BFM), total water content (TW); Clinical Status Assessment Score.

Group 1 patients received nutritional mix Modulen (100-130 g of dry mix), which accounted for 25 % of their daily energy demands, in addition to their basic diet.Group 2 patients

received nutritional mix Peptamen (100-130 g of dry mix), which accounted for 25 % of their daily energy demands, in addition to their basic diet.Group 3 patients received their standard therapy only, as well as the necessary amount of nutrients within a standard diet, based on pre-calculated energy demands.All patients maintained their dietary diaries, which were used to adjust the diet on an individual basis.

The energy demands were calculated using Harris-Benedict formula with adjustment for body mass deficit, taking into account body temperature and activity of a patient.

Energy demands:

$$AEC = EOO*AF*TF*BMD \quad (1)$$

where:AEC — actual energy consumption (kcal/day);

EOO — basal metabolic rate, calculated using Harris-Benedict equations:

EOO (men) = 66 + (13.7*body mass, kg) + (5*height, cm) – (6.8*age)

EOO (women) = 655 + (9.6*body mass, kg) + (1.8*height, cm) – (4.7*age)

AF — activity factor (bed rest: 1.1, movement within room: 1.2, no limitations: 1.3), TF — temperature factor (36–37.0°C: 1.0, 37.1–38.0°C: 1.1, 38.1–39.0°C: 1.2), BMD — body mass deficit (10–20 %: 1.1, 20–30 %: 1.2, >30 %: 1.3).

This study enrolled 120 patients.Males: 18 subjects (45 %) in Group 1, 19 subjects (47.5 %) in Group 2, and 21 subjects (52.5 %) in Group 3.Females: 22 subjects (55 %) in Group 1, 21 subjects (52.5 %) in Group 2, and 19 subjects (47.5 %) in Group 3.

Total duration of CHF history was 15.3±4.3 months, 15.7±4.1 months, and 15.6±4.4 months for Group 1, Group 2, and Group 3, respectively.These characteristics were not significantly different between study groups.

Compensated type 2 diabetes mellitus was reported in 52.5 %, 55 %, and 42.5 % of subjects in Group 1, Group 2, and Group 3, respectively.

Charlson index was > 5 in all groups.

As demonstrated above, there were no statistically significant differences between groups in gender, age, and co-morbidity rates.

In all three groups, decreases of weight and LBM were demonstrated on Day 21 of the treatment period:in Group 1, Day 21 body weight was 56.4±2.6 kg (baseline 66.9±3.5 kg); in Group 2, Day 21 body weight was 56.4±2.1 kg (baseline 66.2±2.9 kg); in Group 3, Day 21 body weight was 55.4±1.7 kg (baseline 67.0±2.9 kg); in Group 1, Day 21 LBM was 40.9±1.7 kg (baseline LBM 47.2±1.9 kg); in Group 2, Day 21 LBM was 42.0±2.0 kg (baseline LBM 47.3±2.0 kg); in Group 3, Day 21 LBM was 41.2±1.9 kg (baseline LBM 46.9±1.8 kg).However, on Day 224, the body weight in Group 1 increased to 62.6±2.7 kg, but was significantly lower than in Group 2 (66.8±2.4 kg) and Group 3 (68.7±1.9 kg).The LBM also increased on Day 224, but it was lower than baseline LBM in Group 1 (44.4±1.6 kg), whereas it reached the baseline in Group 2 (47.4±2.2 kg) and significantly exceeded the baseline in Group 3 (48.1±1.9 kg).

In Group 2 and Group 3, a statistically significant decrease of total fluid content on Day 21 was demonstrated: 34.6±1.8 kg in Group 2 (baseline 47.3±2.1 kg) and 34.4±1.7 in Group 3

(baseline 47.1±1.7 kg).On Day 224, the total fluid content increased in both of these groups: 47.2±2.0 kg and 48.3±1.9, respectively.In Group 1, the total fluid content also decreased by Day 21 (34.3±1.9 kg) compared to baseline (46.8±2.0 kg), but there was no significant increase of the total fluid content on Day 224 (39.3±1.9 kg), unlike in other two groups.

On Day 224, the body fat mass increased significantly in Group 1 to 18.6±1.11 kg (baseline 16,8±1.13 kg), while no significant change in the body fat mass was reported for Group 2 and Group 3:16.8±1.13 kg (baseline 16.5±1.16 kg) in Group 2 and 16.6±1.33 kg (baseline 16.5±1.19 kg) in Group 3.

On Day 21, the BMI decreased from baseline in all groups:21.2±0.60 kg/m^2 in Group 1 (baseline 24.1±0.93 kg/m^2); 21.4±0.59 kg/m^2 in Group 2 (baseline 24.1±0.86 kg/m^2); and 21.5±0.64 kg/m^2 in Group 3 (baseline 24.3±1.01 kg/m^2).On Day 224, the BMI returned to baseline and was 23.7±0.62 kg/m^2 in Group 1, 23.7±0.58 kg/m^2 in Group 2, and 24.5±0.60 kg/m^2 in Group 3.

The body mass increased in all three study groups, but in patients on Modulen this was accountable to increase of muscle and fat mass, and not to increase of total fluid content.This suggests that administration of Modulen improves the nutritional status profile.

Assessment of NT-proBNP, adiponectin, leptin, CRP, IL-6, TNF-α during administration of Modulen and Peptamen added to standard-of-care therapy in CHF patients with NYHA class III-IV.

The level of NT-proBNP in all groups was over 3000 pg/mL.

Reduction of chronic inflammation intensity was demonstrated in patients receiving Modulen:on Day 224, there was a decrease in levels of CRP (4.7±0.4 mg/ml vs. baseline 8.9±0.7 mg/mL), IL-6 (5.2±0.4 U/L vs. baseline 11.8±0.8 U/L), TNF-α (3.4±0.2 U/L vs. baseline 6.8±0.4 U/L).Levels of adiponectin also declined in patients on Modulen: 15.8±1.5 μg/mL vs. baseline 24.4±1.5 μg/mL. No significant changes were demonstrated in Group 2 and Group 3.

There were no significant changes of leptin levels in any of the groups.

Assessment of hospitalization events showed the following results.In Group 1, during treatment with Modulen, 32 hospitalization events were reported per year: 22 hospitalizations were due to CHF decompensations, in 12 of these cases congestive pneumonia was also present; 2 events were due to myocardial infarctions; 5 events were for hypertensive crisis, with 2 of them progressing to CVA; 3 events were due to fibrillation paroxysms. Per-patient hospitalization rate was 0.55±0.01 event/person.There were 5 deaths over one year of observation in Group 1:2 deaths were caused by CHF decompensation, 2 deaths were caused by CVA, 1 death was caused by AMI.

In Group 1, during treatment with Peptamen, 38 hospitalization events were reported over one year of observation:of these, 27 hospitalization events were due to CHF decompensations, with 15 cases accompanied by congestive pneumonia; 3 events were due to acute myocardial infarctions; 7 events were due to hypertensive crisis, 1 of them progressed to CVA; 4 events were due to fibrillation paroxysms. Per-patient hospitalization rate was 0.95±0.02 event/person.There were 8 deaths over one year of observation in Group 2:5 deaths were caused by CHF decompensation, 2 deaths were caused by AMI, 1 death was caused by CVA.

In Group 3, 48 hospitalizations were reported over one year of observation: 41 events were due to CHF decompensations, in 22 of which congestive pneumonia was also present; 2 events were due to acute myocardial infarctions; 4 events were for hypertensive crisis, with 2 cases progressing to CVA; 1 event was due to a fibrillation paroxysm.Per-patient hospitalization rate was 1.2±0.03 event/person.There were 12 deaths over one year of observation in Group 3:8 deaths were caused by CHF decompensation, 2 deaths were caused by AMI, 2 deaths were caused by CVA.

4. Conclusion

Rates of hospitalization events and deaths over one year were lower in subjects receiving Modulen compared to Peptamen and standard-of-care therapy.

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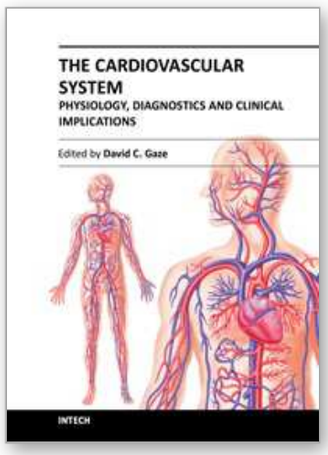

The Cardiovascular System - Physiology, Diagnostics and Clinical Implications

Edited by Dr. David Gaze

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The cardiovascular system includes the heart located centrally in the thorax and the vessels of the body which carry blood. The cardiovascular (or circulatory) system supplies oxygen from inspired air, via the lungs to the tissues around the body. It is also responsible for the removal of the waste product, carbon dioxide via air expired from the lungs. The cardiovascular system also transports nutrients such as electrolytes, amino acids, enzymes, hormones which are integral to cellular respiration, metabolism and immunity. This book is not meant to be an all encompassing text on cardiovascular physiology and pathology rather a selection of chapters from experts in the field who describe recent advances in basic and clinical sciences. As such, the text is divided into three main sections: Cardiovascular Physiology, Cardiovascular Diagnostics and lastly, Clinical Impact of Cardiovascular Physiology and Pathophysiology.

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